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Techniques, perspectives, and challenges of bioactive peptide generation: A comprehensive systematic review

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Abstract

Due to the digestible refractory and absorbable structures of bioactive peptides (BPs), they could induce notable biological impacts on the living organism. In this regard, the current study was devoted to providing an overview regarding the available methods for BPs generation by the aid of a systematic review conducted on the published articles up to April 2019. In this context, the PubMed and Scopus databases were screened to retrieve the related publications. According to the results, although the characterization of BPs mainly has been performed using enzymatic and microbial *in-vitro* methods, they cannot be considered as suitable techniques for further stimulation of digestion in the gastrointestinal tract. Therefore, new approaches for both *in-vivo* and *in-silico* methods for BPs identification should be developed to overcome the obstacles that belonged to the current methods. The purpose of this review was to compile the recent analytical methods applied for studying various aspects of food-derived biopeptides, and emphasizing generation at *in vitro*, *in vivo*, and *in silico*.

KEYWORDS

bioactive peptides, digestion, hydrolysis, peptide identification, peptidomics, systematic review

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1 | INTRODUCTION

Bioactive peptides (BPs) similar to digestion refractory and absorbable peptides can induce potentially biological functions (Chakrabarti, Guha, & Majumder, 2018; Sultan, Huma, Butt, Aleem, & Abbas, 2018), while the beneficial effects of BPs were well demonstrated (El-Fattah, Sakr, El-Dieb, & Elkashef, 2017; Barati et al., 2017; Orona-Tamayo, Valverde, & Paredes-Lopez, 2019) such as anticancer (Díaz-Gómez, Castorena-Torres, Preciado-Ortiz, & García-Lara, 2017), antihypertensive (Aluko, 2015; Bhat, Kumar, & Bhat, 2017), antimicrobial (Miao et al., 2016), cholesterol lowering (Nagaoka, 2019), and antidiabetics properties (Admassu, Gasmalla, Yang, & Zhao, 2018). Although some BPs freely exist in their natural form, most of them are known to be released from the main proteins through enzymatic or microbial digestions (Sánchez & Vázquez, 2017). Currently, more than 3,000 different BP have been reported in BIOPEP-UMW database. BPs are organic compounds formed by amino acids (AAs) joined by peptide bonds. The AA sequence determines the function of the BPs, once at the time that the peptides are released from the parent protein where they are encrypted. Many BPs share some structural features including a peptide residue length between 2 to 20 AAs, and the presence of hydrophobic AAs in addition to arginine, lysine, or proline residues (Sánchez & Vázquez, 2017).

In vitro conditions were used in most of the previous studies to characterize the BPs. In this regard, the enzymatic hydrolysis or fermentation of food proteins are the two other common methods of BPs identification (X. Wang, Yu, Xing, & Li, 2017), while at a specified temperature and pH, protein substances are subjected to enzymatic or microbial hydrolysis (Najafian & Babji, 2014; Udenigwe & Aluko, 2012). The *in vitro* process for BPs isolating involves protein selection, enzymatic hydrolysis, separation, and purification, while after the last step, the biological activity, peptide sequences, peptide structure, and the corresponded functional properties were determined (Daliri, Oh, & Lee, 2017). Due to digestion or unabsorbability of the peptides through the gastrointestinal tract (GIT), the *in-vitro* characterization of BPs could not demonstrate their *in-vivo* activities.

Different GIT proteases including pepsin (EC 3.4.4.1), trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), brush-border, and serum peptidases can affect the peptide bonds through endogenous cleavages, which can result in the modification of the structure and also in loss of the biological functions. In this context, most of the entered BPs in the duodenum and jejunum cannot be absorbed into the bloodstream (Foltz, van der Pijl, & Duchateau, 2010). Digestion resistance and absorbability as the two main aspects of BPs function should be evaluated before performing further characterization of BPs in the blood following food ingestion (J. Chen et al., 2018; Herregods et al., 2011). Therefore, to solve the abovementioned issue, some *in-vivo* and *in-silico* methods were developed for BPs production. However, each one of the *in-vivo*- and *in-silico*-based methods poses its own disadvantages. In this regard, the current review aimed to provide the required insights regarding the methods of BPs identification, by emphasizing on *in-vivo*- and *in-silico*-based methods.

NOMENCLATURE: AAs, Amino acids; ABTS, 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic; ACE inhibitors, Angiotensin-converting enzyme inhibitors; AISC, Aspiration of intestinal and stomach contents; BPs, Bioactive peptides; DPP-IV, Dipeptidyl peptidase IV; GIP, Glucose-dependent insulinotropic polypeptide; GIT, Gastrointestinal tract; GLP-1, Glucagon-like peptide-1; HCC, Hepatocellular carcinoma; HPLC-MALDI-TOF MS/MS, High-performance liquid chromatography-matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry/mass spectrometry; LC MS/MS LTQ-Orbitrap, Liquid chromatography mass spectrometry/mass spectrometry linear trap quadropole orbitrap; LC-ESI MS/MS, Liquid chromatography coupled with electrospray ionization mass spectrometry/mass spectrometry; LC-ESI-QTOF-MS, Liquid chromatography coupled with electrospray ionization quadrupole time of flight mass spectrometry; LC-MS/MS, Liquid chromatography–mass spectrometry/mass spectrometry; LMW peptides, Low molecular weight peptides; MALDI-TOF/TOF, Matrix-assisted laser desorption/ionization-time-of-flight; MRM, Multiple reaction monitoring; MS, Mass spectrometry; NCBI, National Center for Biotechnology Information; PAC, Postabsorption characterization; PB, Proteome based; PRISMA, Preferred reporting items for systematic reviews and meta-analyses; QSAR, Quantitative structure-activity relationships; RAS, Renin–Angiotensin system; RCTs, Randomized clinical trials; RP-HPLC, Reversed-phase high-performance liquid chromatography; SCF, Standard curve fitting; SHR, Spontaneously hypertensive rats; SPPS, Solid-phase peptide synthesis; SPS, Solution phase synthesis; TFA, Trifluoroacetic.

2 | METHODOLOGY

The recommended guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were used for collecting the related data (Fakhri et al., 2019; Khaneghah, Fakhri, & Sant'Ana, 2018) (Table 1). In this regard, a search strategy was conducted to retrieve the related citations considering the methods of BPs production among some international databases such as PubMed and Scopus with the terms of "(bioactive peptide[Title/Abstract]) OR (bio-active peptide[Title/Abstract]) OR (bioactive peptide[Title/Abstract]) OR (food-derived peptide[Title/Abstract]) OR (food-derived peptide[Title/Abstract]) OR (food-derived bioactive peptide[Title/Abstract]) OR (food-derived bioactive peptide[Title/Abstract]) OR (collagen hydrolysate[Title/Abstract])" up to April 30, 2019. Moreover, the bibliographies of retrieved citations were cross-referenced to achieve some additional articles. The conference proceedings, abstracts, posters, and

TABLE 1 Main characteristics of the included the studies regarding *in-silico* methods for BPs production

Peptide origin	Enzyme (s) used	Peptide sequence	activity	Confirmatory method/confirmed fragments	Ref.
κ-casein variants	pepsin, trypsin, chymotrypsin A, chymotrypsin C, pancreatic elastase I, pancreatic elastase II, oligopeptidase B, and oligopeptidase II	Ile-Arg (**IC_{50}: 695.0** μM), Arg-Phe (**IC_{50}: 93.0** μM), Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg (**IC_{50}: 132.5** μM), Ala-Ile-Pro (**IC_{50}: 690.0** μM), Val-Thr-Ser-Thr-Ala-Val (**IC_{50}: 52.0** μM), Pro-Ser-Tyr (**IC_{50}: 16.0** μM), Ile-Ala-Lys (**IC_{50}: 15.7** μM)	ACE inhibitor	–/–	(Weimann, Meisel, & Erhardt, 2009)
Egg	Thermolysin+ pepsin	Ile-Arg-Try (**IC_{50}: 0.59** μM), Leu-Lys-Pro (**IC_{50}: 1.4** μM), Ile-Gln-Try (**IC_{50}: 2.8** μM)	ACE inhibitor	Enzymatic *In-vitro* / **Ile-Arg-Try**-Cys-Thr, **Leu-Lys-Pro**-Ile, **Ile-Gln-Try**-Cys-Ala	(Majumder & Wu, 2010)
Cereal RuBisCO	Thermolysin	Phe-Cys, Phe-Arg and Phe-Gly	ACE inhibitor (**A: 0.4295**), DPP-IV Inhibitor (**A: 0.0758**), antioxidative (**A: 0.0568**), UbMP activating (**A: 0.0211**)	–/–	(Udenigwe, Gong, & Wu, 2013)
	Papain	Pro-Trp-Gly, Phe-Arg and Trp-Arg			
Meat	Papain+ Ficain+ Bromelain	IIY (**ACE IC_{50}: 1.08** μM), DFY (**ACE IC_{50}: 43.35** μM), PPG (**DPP-IV IC_{50}: 2252.68** μM)	ACE inhibitor, DPP-IV Inhibitor	–/–	(Lafarga, O'Connor, & Hayes, 2014)
Serum albumin	Trypsin+ chymotrypsin+ pepsin	IA, LF, PR, LY, QK, AW, EK, GM, EY, AR, EK, KP, VR, VPK, VE, VK, AA	—	–/–	(Dave, Montoya, Rutherfurd, & Moughan, 2014)
β-Lactoglobulin	Trypsin	IVTQTMK, VAGTWYS, LR, LKPTPEGD (**IC_{50} < 100** μM)	DPP-IV Inhibitor	–/–	(Tulipano, Faggi, Nardone, Cocchi, & Caroli, 2015)
	Trypsin+ chymotrypsin	IVTQTMK, VAGTW (**100 < IC_{50} < 200** μM), LKPTPEGD (**IC_{50} < 100** μM), IPAV (**IC_{50} < 100** μM)			
α-Lactalbumin	Trypsin	LK, WLAHK (**200 < IC_{50} < 500** μM), LAHK			
	Trypsin+ chymotrypsin	LK, LAHK, ILKD			
Whey protein	Trypsin	CLLLALGLALACGAQAIIVTQTMK, VAGTWYSLAMAASDISLLDAQS-APLR	ACE inhibitor	Enzymatic *In-vitro* / IIVTQTMK, SLAMAASDISLL-DAQSAPLR	(Chatterjee, Kanawjia, Khetra, & Saini, 2015)

(Continues)

TABLE 1 (Continued)

Peptide origin	Enzyme (s) used	Peptide sequence	activity	Confirmatory method/confirmed fragments	Ref.
Meat	Pepsin+ trypsin+ chymotrypsin	PPL (**IC_{50}: 2.86** mM), APPH (**IC_{50}: 3.95** mM), IPP (**IC_{50}: 4.02** mM), PPG (**IC_{50}: 2.70** mM)	PEP inhibitor	–/–	(Lafarga, O'Connor, & Hayes, 2015)
Oryzacystatin 1 (**A for ACE: 0.4298; A for DPP-IV: 0.0789**) and Oryzacystatin 2 (**A for ACE: 0.4172; A for DPP-IV: 0.0760**)	Thermolysin and pepsin	HL, GGE, RF, KL, KE, MA, PA, RA	ACE inhibitor, DPP-IV Inhibitor	–/–	(Udenigwe, 2016)
Patatin	Chymotrypsin C	RY, IY, FP, GP, RW, GQ, GE, KL	ACE inhibitor	–/–	(Fu, Wu, Zhu, & Xiao, 2016)
	Papain	PR, LA, DA, WG, SG, LG			
	Pepsin	RL, KW, RW, DA, GQ, GE			
	Proteinase K	RY, GP, RW, AI			
	Ficain	RL, PRY, IG, DA, WG, SG, TG			
rice bran proteins (Glutelin type-A1 [A_E: 0.0295; W: 0.1065]; Glutelin type-B1 [A_E: 0.0327; W: 0.1164]; Glutelin type-C [A_E: 0.0345; W: 0.1198]; Globulin 1 [A_E: 0.0231; W: 0.0883]; Globulin 2 [A_E: 0.0135; W: 0.0555]; Prolamin [A_E: 0.0153; W: 0.0887])	Papain	RL, GE, TF, GA, VK, AH, ITT, GDAP	ACE inhibitor	–/–	(Rani et al., 2017)

(Continues)

TABLE 1 (Continued)

Peptide origin	Enzyme (s) used	Peptide sequence	activity	Confirmatory method/confirmed fragments	Ref.
Rice bran derived globulin 1 (*A* for ACE: 0.3197; *A* with DPP-IV: 0.5164) and globulin 2 (*A* for ACE: 0.3058; *A* for DPP-IV: 0.4175)	Pepsin	IL, NA, SY, SF, HA	DPP-IV inhibitory	–/–	(Rani et al., 2017)
	Bromelain	HA, NA, NG, LA, NG			
	Ficain	EG, IL, IN, EK, FN			
	Papain	VR, NA, WE, DR			
Camel milk	Trypsin	VLVLDTDYK (**IC_{50}: 424.4 μM**), VAGTWY (**IC_{50}: 174.0 μM**), TPEVDDEALEK (**IC_{50}: 319.5 and 587.7 μM**), LPVPQ (**IC_{50}: 43.69 μM**)	DPP-IV inhibitory	Enzymatic *In-vitro* / VLVLDTDYK, VAGTWY, TPEVDDEALEK, LPVPQ	(Nongonierma, Paolella, Mudgil, Maqsood, & FitzGerald, 2017)
Goat milk	Pepsin (α-casein S1 [A_E: 0.0545; *W*: 0.1275]; α-casein S2 [A_E: 0.0611; *W*: 0.1666]; β-casein [A_E: 0.0614; *W*: 0.1386]) and chymotrypsin A (α-casein S1 [A_E: 0.0273; *W*: 0.0639]; α-casein S2 [A_E: 0.0175; *W*: 0.0477]; β-casein [A_E: 0.0219; *W*: 0.0497])	RL, RF, GY, AY, PL, VY, DKIHPF, PL	ACE inhibitor	–/–	(Rani et al., 2017)

(Continues)

TABLE 1 (Continued)

Peptide origin	Enzyme (s) used	Peptide sequence	activity	Confirmatory method/confirmed fragments	Ref.
Yak milk	Chymotrypsin (*A* for ACE: 0.1104), trypsin (*A* for ACE: 0.0703), proteinase K (*A* for ACE: 0.1750), thermolysin (*A* for ACE: 0.1573), papain (*A* for ACE: 0.1608), alcalase (*A* for ACE: 0.0969)	PPEIN, PLPLL, PPEINT	ACE inhibitor	–/–	(Lin et al., 2018)
Meat	Trypsin, chymotrypsin, pancreatic elastase	EG, SG, HG, MG, PEL, SG, TV	—	AISC / EG, SG, HG, MG, PEL, SG, TV	(Sayd et al., 2018)
Bambara bean protein	thermolysin	AR, VK, AR, VK, FG	ACE inhibitor	Enzymatic *In-vitro* / AR, VK, AR, VK, FG	(Mune Mune, Minka, & Henle, 2018)
Tuber storage proteins	Trypsin+ chymotrypsin+ pepsin	MY, GPL, VAY, AF, IR	ACE inhibitor, DPP-IV inhibitory	–/–	(Ibrahim, Bester, Neitz, & Gaspar, 2019)
Honeybee pupae	Trypsin+ chymotrypsin+ pepsin	AVPFSIVGR, PGKVHIT (**IC_{50}: 223.869 μM**)	ACE inhibitor	Enzymatic *In-vitro* / AVFPSIVGR, PPVLVFV, PGKVHIT	(Yang, Chen, Liu, Zhang, & Luo, 2019)
Green Macroalga Ulva lactuca	Papain	SGAASASGAA, ATKPAN	—	Enzymatic *In-vitro* / SGAASASGAA, ATKPAN, VVPKAAPPPN	(Garcia-Vaquero, Mora, & Hayes, 2019)
Oilseed proteins	Subtilisin, pepsin (pH = 1.3), pepsin (pH > 2)	MW, AW, PSF, SPF, ACQCL	DPP-IV inhibitory	–/–	(Han, Maycock, Murray, & Boesch, 2019)

Abbreviations: AISC, aspiration of intestinal and stomach contents method; BPs, bioactive peptides; DPP-IV, dipeptidyl peptidase-4; ACE, angiotensin-converting enzyme; A, alanine; R, arginine; N, asparagine; D, aspartic acid; C, cysteine; E, glutamic acid; Q, glutamine; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine; Phe, phenylalanine; Hyp, hydroxy prolin; Ile, isoleucine; Leu, leucine; Pro, proline; Gly, glycine; Ala, alanine; Glu, glutamic acid; Hyp-peptides, hydroxy proline containing peptides.

IC50: The half maximal inhibitory concentration.

A: The frequency of BPs occurrence in a protein.

A_E: The frequency of release of BPs with a given activity by selected enzymes.

W: The relative frequency of release of fragments with a given activity by selected enzymes.

book chapters due to lack of peer review and review articles based on the recommended criteria were also excluded (Atamaleki et al., 2019; Fakhri et al., 2019; Khaneghah et al., 2019; Khaneghah, Fakhri, Gahruie, Niakousari, & Sant'Ana, 2019; Khaneghah et al., 2019).

2.1 | Selection of the studies, data extraction, and management

At first, all papers were collected and then entered to end note. After that, screening the papers was performed based on their titles and abstracts. Consequently, full texts were reviewed to be screened in terms of the inclusion/exclusion criteria (Fakhri et al., 2019). The relevant data were extracted including first author, year of publication, type of study, the proposed method for BPs production, and the BPs amino acid sequence.

2.2 | Data synthesis

A narrative synthesis method was used for analyzing the data extracted (Popay et al., 2006). The confidence in the accumulated body of evidence was qualitatively assessed, but it was not graded.

3 | RESULTS

In the primary step, 17,722 articles were obtained while after screening their title/abstract, 911 papers were included, and after full-text evaluation, only 786 articles were included, which can be divided into *in-silico* ($n = 22$), *in-vitro* ($n = 728$), and *in-vivo* ($n = 36$). Each category itself has been divided into some specific subgroups. The PRISMA flowchart diagram comprehensively illustrated the included article, categories, and subgroups (Figure 1). Also, the review results have been summarized in Tables 1 and 2.

4 | DISCUSSION

After performing a systematic search on PubMed and Scopus, three different methods for BPs generation were defined. The *in-vitro* method including microbial and enzymatic techniques and the *in-vivo* method including postabsorption characterization (PAC), aspiration of intestinal and stomach contents (AISCs), and standard curve-fitting (SCF) methods have been used to identify the BPs. Also, over the last decade, *the in-silico* method has been proposed to predict the BPs structure before *performing the in-vitro* or *in-vivo* assays. The *in-silico* method itself consists of proteome based and genome-based methods.

4.1 | *In-silico* methods

4.1.1 | Proteome-based method

Recently, proteome based *in-silico* method was used to predict new types of BPs (Liao, Jahandideh, Fan, Son, & Wu, 2018). In this approach, the sequences of the desired food item's major proteins are obtained from the databases such as National Center for Biotechnology Information (NCBI) protein database (http://www.ncbi.nlm.nih.gov) (Rani, Pooja, & Pal, 2017; Sayers et al., 2019) or UniProtKB database (https://www.uniprot.org) (Lin et al., 2018). UniProtKB database contains two parts named as SwissProt (https://www.uniprot.org/uniprot/?query) and TrEMBL (https://www.uniprot.org/uniprot/?query=reviewed:no). After the sequence extraction, *in-silico* digestion was performed by software. The Peptidecutter software (http://www.expasy.ch/tools/peptidecutter/), available at ExPASy Molecular Biology Server (Gasteiger et al., 2005), and BIOPEP-UWM ''enzyme action'' tool (Minkiewicz, Iwaniak, & Darewicz, 2019) are mostly among the most common software used for *the in-silico* digestion. The abovementioned two types of the software comprise a range of proteases, while some of which are not specific to the human, and some others are from bacteria. Then, specific enzymes or chemicals were selected, individually or in combination, depending on the aim of research/study. After *in-silico* proteolysis, the released peptides with the reported bioactivity were identified using existing databases such as NeuroPep (Y. Wang et al., 2015), MAHMI (Blanco-Míguez, Gutiérrez-Jácome, Fdez-Riverola, Lourenço, & Sánchez, 2017), BIOPEP-UWM (Minkiewicz et al., 2019), and antihypertensive peptide database (AHTPDB) (Kumar et al., 2015). In addition to the earlier mentioned databases, some other databases such as PepBank (Shtatland, Guettler, Kossodo, Pivovarov, & Weissleder, 2007), PeptideDB (http://www.peptides.be/), BitterDB (Wiener, Shudler, Levit, & Niv, 2012), CAMP (Waghu, Barai, Gurung, & Idicula-Thomas, 2016), and APD (G. Wang, Li, & Wang, 2016) can be also used.

BIOPEP-UWM database (Minkiewicz et al., 2019) contains 3,790 known BPs with 48 biological activities. The potential BPs prediction of the proteins was made using the above-mentioned databases. BIOPEP-UWM not only interlinks databases of peptide, protein, and BPs sequences, but also aids in the prediction of allergenic peptides (Rani, Pooja, & Pal, 2018). The other peptides were investigated for the possibility of exhibiting the bioactivity using PeptideRanker (Mooney, Haslam, Pollastri, & Shields, 2012). Many novel peptides without any reported function can be achieved from the proteins, and then PeptideRanker evaluated the likelihood of peptide bioactivity by assigning the scores of 0 and 1, where"0″ represents the least and "1″represents the most likely to be BPs (Rani et al., 2017).

TABLE 2 The studies used *in-vivo* methods for BPs production*

Method	subject	Peptide origin	Peptide sequence OR peptide name	activity	Reference
AISC	Calf	Milk	YQEPVLQPVRGPFPIIV, DAYPSGAW, AYFYPEL, RYLGYL	—	(Yvon & Pelissier, 1987)
AISC	Mini-pigs	Milk	β-casein 60 to 70, α_{s1}-casein 66 to 74	—	(Meisel & Frister, 1989)
PAC	Human	Milk	MAIPPKKNQDK, IAIPPKKIQDK	Antithrombotic	(Chabance et al., 1995)
AISC	Human	Milk	MAIPPKKNQDKT, AIPPKKNQDKTE	Antithrombotic	(Chabance et al., 1998)
SCF	Human	Sardine muscle	Val-Tyr	Antihypertensive	(Matsui et al., 2002)
SCF	Human	Collagen	Ala-Hyp, Ala-Hyp-Gly, Pro-Hyp-Gly, Leu-Hyp, Ile-Hyp, Phe-Hyp	—	(Iwai et al., 2005)
AISC	Pig	Beef meat and trout flesh	AGDDAPRAVF, AGFAGDDAPR, AGDDAPRAVF, AGFAGDDAPR	—	(Bauchart et al., 2007)
SCF	Rat	Milk	Ile-Pro-Pro	Antihypertensive	(Jauhiainen et al., 2007)
SCF	Human	Gelatin	Ala-Hyp, Leu-Hyp, Ile-Hyp, Phe-Hyp, Pro-Hyp-Gly, Ala-Hyp-Gly, Ser-Hyp-Gly	—	(Ohara, Matsumoto, Ito, Iwai, & Sato, 2007)
SCF	Human	Soy	[a]Lunasin	anti-inflammatory	(Dia, Torres, De Lumen, Erdman, & Gonzalez De Mejia, 2009)
SCF	Human	Collagen	Pro-Hyp, Leu-Hyp, Pro-Hyp-Gly, Phe-Gly, Ala-Hyp, Hyp-Gly, Glu-Hyp-Gly, Ala-Hyp-Gly, Ser-Hyp-Gly	Anti-hypertensive	(Iwai et al., 2009)
SCF	Human	Collagen	Ala-Hyp-Gly, Ser-Hyp-Gly, Ala-Hyp, Phe-Hyp, Leu-Hyp, Ile-Hyp, Gly-Pro-Hyp, Pro-Hyp-Gly	—	(Ichikawa et al., 2010)
SCF	Rat	Collagen	Gly-Pro-Hyp	Anti-hypertensive	(Watanabe-Kamiyama et al., 2010)
SCF	Rat	Collagen	Pro-Hyp, Hyp-Gly	—	(Sugihara, Inoue, Kuwamori, & Taniguchi, 2012)
SCF	Rat	Collagen	Pro-Hyp	—	(Kawaguchi, Nanbu, & Kurokawa, 2012)
SCF	Human	Elastin	Pro-Gly	—	(Shigemura et al., 2012)
AISC	Piglets	Infant formula	k-casein 155 to 161, k-casein 155 to 160, k-casein 115 to 121	—	(Bouzerzour et al., 2012)
AISC	Mini pigs	Milk	FQSEEQQQTEDELQDK, IEKFQSEEQQQTEDE	—	(Barbé et al., 2014)
SCF	Human	Collagen	Hyp containing peptides	—	(Shigemura, Kubomura, Sato, & Sato, 2014)
SCF	Human	Collagen	Pro-Hyp-Gly, Pro-Hyp	—	(Taga, Kusubata, Ogawa-Goto, & Hattori, 2014)

(Continues)

TABLE 2 (Continued)

Method	subject	Peptide origin	Peptide sequence OR peptide name	activity	Reference
SCF	Pig	Milk	Ile-Pro-Pro, Leu-Pro-Pro, Val-Pro-Pro	Antihypertensive	(Ten Have, van der Pijl, Kies, & Deutz, 2015)
SCF	Human	Milk	Ile–Trp, Trp–Leu	Antihypertensive	(Kaiser et al., 2016)
SCF	Human	Collagen	Gly-Pro-Hyp, Pro-Hyp, Hyp-Gly	—	(Yamamoto, Deguchi, Onuma, Numata, & Sakai, 2016)
SCF	Mice	Collagen	Glu-Hyp-Gly	—	(Taga, Kusubata, Ogawa-Goto, & Hattori, 2016)
SCF	Rat	Collagen	Gly-Pro-Hyp, Pro-Hyp	—	(Sontakke, Jung, Piao, & Chung, 2016)
AISC	Rat	Milk	DIDGYGGIALPEL, NESTEYGLFQI, GPPVSCIKRDSPIQC, KRDSPI	Immuno-regulatory and Antithrombotic	(Wada, Phinney, Weber, & Lönnerdal, 2017)
SCF	mice	Collagen	Gly-Pro-Hyp, Pro-Hyp	Skin health	(Yazaki et al., 2017)
SCF	mice	Collagen	Cyclo Ala-Hyp, Cyclo Leu-Hyp	—	(Taga, Kusubata, Ogawa-Goto, & Hattori, 2017)
AISC	Mini pigs	Meat	GRLQTESGEFS, IQGTLEDQI-ISANPLLEAFGNAK	—	(Sayd et al., 2018)
SCF	Human	Collagen	Pro-Hyp-Gly, Pro-Hyp, Ile-Hyp, Leu-Hyp, Hyp-Gly, Glu-Hyp, Ala-Hyp	—	(Shigemura et al., 2018)
SCF	Human	Collagen	Cyclic Pro-Hyp	—	(Shigemura et al., 2018)
SCF	Human	Collagen	Ala-Hyp-Gly, Pro-Hyp-Gly, Ser-Hyp-Gly	—	(Asai et al., 2019)
SCF	Rat	Collagen	Gly-Pro, Pro-Hyp	Skin health	(Lee et al., 2019)
SCF	Rat	Walnut	VEGNLQVLRPR, HNLDTQTESDV	—	(Xiaoyu Yang et al., 2019)
AISC	Mice	Casein	AVPYPQR	Anticoagulant	(Tu et al., 2019)
SCF	Human	Collagen	Gly-Pro-Hyp, Ala-Hyp-Gly, Glu-Hyp-Gly	—	(Taga, Iwasaki, Shigemura, & Mizuno, 2019)

AISC, aspiration of intestinal and stomach contents method; BPs, bioactive peptides; PAC, postabsorption characterization; SCF, standard curve fitting method; A, alanine; R, arginine; N, asparagine; D, aspartic acid; C, cysteine; E, glutamic acid; Q, glutamine; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine; Phe, phenylalanine; Hyp, hydroxy prolin; Ile, isoleucine; Leu, leucine; Pro, proline; Gly, glycine; Ala, alanine; Glu, glutamic acid; Hyp-peptides, hydroxy proline containing peptides.
[a]Lunasin: 43-aminoacide peptide isolated from soy.

The predicted BPs should be examined in terms of the physicochemical properties, allergenicity, and toxicity. ProtParam (Gasteiger et al., 2005) and ToxinPred (Gupta et al., 2013) were used to assess the physicochemical properties and toxicity of the peptides, respectively. For allergenicity assessment, the peptides are usually evaluated by Allerdictor (Dang & Lawrence, 2014) and AllerTOP (Dimitrov, Bangov, Flower, & Doytchinova, 2014) *in silico*. *In-silico* approach has diverse aspects in the field of BPs research, and here we only talked about BPs production (Blanco-Míguez, Fdez-Riverola, Lourenço, & Sánchez, 2019; Tu, Feng, et al., 2017). Kalmykova et al. (2018) and Tu, Cheng, Lu, and Du (2018) in two different review articles comprehensively described the websites of databases and bioinformatics tools for *in-silico* analysis of the BPs (Table 3).

After *in-silico* digestion and BPs mining, several parameters have been used to characterize the proteins as the potential precursors of the BPs. The frequency of BPs occurrence in a protein sequence, potential bioactivity of protein-derived fragments, the frequency of fragments released with a given function by selected enzymes, and the relative frequency of fragments released with a given function by selected enzymes were mostly used (Minkiewicz et al., 2019). The parameters are widely described in Table 4.

TABLE 3 *In silico* tools used for discovery of bioactive peptides from food proteins

Databases and tools	Website	Reference	Application
UniProtKB	http://www.uniprot.org/	(Lin et al., 2018)	protein sequences
NCBI Protein	http://www.ncbi.nlm.nih.gov/protein	(Rani et al., 2017; Sayers et al., 2019)	
BIOPEP-UWM	http://www.uwm.edu.pl/biochemia/	(Minkiewicz et al., 2019)	
PepBank	http://pepbank.mgh.harvard.edu/	(Shtatland et al., 2007)	
BioPD	http://biopd.bjmu.edu.cn/	(Shi et al., 2004)	
SwePep	http://www.swepep.org/	(Falth et al., 2006)	
EROP-Moscow	http://erop.inbi.ras.ru/	(Zamyatnin, Borchikov, Vladimirov, & Voronina, 2006)	
MilkAMP	http://milkampdb.org/	(Théolier, Fliss, Jean, & Hammami, 2014)	
PeptideDB	http://www.peptides.be/	(Wiener et al., 2012)	
AMPer	http://marray.cmdr.ubc.ca/cgi-bin/amp.pl	(Fjell, Hancock, & Cherkasov, 2007)	
BIOPEP-UWM	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	(Minkiewicz et al., 2019)	Databases of proteolytic enzymes and *in silico* digestion platforms
PeptideCutter	http://web.expasy.org/peptide_cutter/	(Gasteiger et al., 2005)	
PoPS	http://pops.csse.monash.edu.au/pops-cgi/index.php	(Boyd, Pike, Rudy, Whisstock, & Garcia de la Banda, 2005)	
Enzyme Predictor	http://bioware.ucd.ie/~enzpred/Enzpred.php	(Vijayakumar et al., 2012)	
PeptideRanker	http://bioware.ucd.ie/~compass/biowareweb/		Prediction of potential bioactivity
BIOPEP-UWM	http://www.uwm.edu.pl/biochemia/index.php/en/biopep	(Minkiewicz et al., 2019)	
AntiBP2	http://www.imtech.res.in/raghava/antibp2/	(Lata, Mishra, & Raghava, 2010)	
PeptideLocator	http://bioware.ucd.ie/	(Mooney, Haslam, Holton, Pollastri, & Shields, 2013)	
ToxinPred	http://www.imtech.res.in/raghava/toxinpred/	(Gupta et al., 2013)	Toxicity/allergenicity prediction
AlgPred	http://www.imtech.res.in/raghava/algpred/	(Saha & Raghava, 2006)	
Allerdictor	http://allerdictor.vbi.vt.edu/	(Dang & Lawrence, 2014)	
EPIMHC	http://bio.dfci.harvard.edu/epimhc/	(Reche, Zhang, Glutting, & Reinherz, 2005)	
ProPeppe	https://propepper.net/	(Juhász, Haraszi, & Maulis, 2015)	
SORTALLER	http://sortaller.gzhmu.edu.cn/	(Zhang et al., 2012)	

Molecular docking was widely used for drug discovery. In the research field of BPs, these models were also used widely. Molecular docking of BPs with their targets such as angiotensin-converting enzyme (ACE) (EC: 3.4.15.1), renin (EC: 3.4.23.15), or dipeptidyl peptidase IV (DPP-IV) (EC: 3.4.14.5) could propose similar structures with higher efficacy and affinity to their targets (Tu, Cheng, et al., 2018; Tu, Wang, et al., 2018). Novel designed peptides of sequences Thr-Ala-Trp, Val-Lys-Trp, Lys-Tyr-Trp, and Tyr-Ala-Trp were derived from the peptides Thr-Ala-Tyr, Tyr-Ala, Lys-Tyr, and Val-Lys that were identified from a hydrolysate of oyster, and increased the peptides potency compared to their corresponding parental fragments (Xie, Kim, Ha, Choung, & Choi, 2014).

Although *in-silico* method is considered as easy and cost-saving for identification of BPs, it should be validated by *in-vitro* or *in-vivo* methods. Majumder et al. (2010) in an *in-silico* approach predicted **Ile-Arg-Tyr**, **Leu-Lys-Pro**, and **Ile-Gln-Tyr** as potent ACE inhibitors originated from the egg proteins. Surprisingly, after pretreatment of the protein by reducing the agents or sonication and *in-vitro* digestion with pepsin and thermolysin, **Ile-Arg-Tyr**-Cys-Thr,

TABLE 4 The parameters characterizing proteins as potential precursors of BPs

Parameter	Formula	Reference
The frequency of BPs occurrence in a protein (A)	$A = \frac{a}{N}$ a: the number of fragments with a given activity; N: The number of amino acid residues	(Minkiewicz, Dziuba, & Michalska, 2011)
The potential bioactivity of protein fragments (B)	$B = \frac{[\Sigma (\frac{a_i}{EC_{50i}})]}{N}$ or $B = \frac{[\Sigma (\frac{a_i}{IC_{50i}})]}{N}$ a_i: the number of bioactive fragment in a protein sequence. EC_{50i}: the concentration of the BP corresponding to its half-maximal activity. IC_{50i}: the concentration of the BP corresponding to half-maximal inhibition. N: the number of AA residues	(Minkiewicz et al., 2011)
The frequency of release of BPs with a given activity by selected enzymes (A_E)	$A_E = \frac{d}{N}$ d: the number of peptides with a given activity (e.g., ACE inhibitors) released by a given enzyme.N: The number of amino acid residues in protein	(Minkiewicz et al., 2019)
The relative frequency of release of fragments with a given activity by selected enzymes (W)	$W = \frac{A_E}{A}$	(Minkiewicz et al., 2019)

Abbreviation: BPs, bioactive peptides.

Leu-Lys-Pro-Ile, and **Ile-Gln-Tyr**-Cys-Ala were characterized as the ACE inhibitors. Although the sequences achieved from *in-silico* and *in-vitro* assays were not thoroughly similar, the characterized BPs contained the sequences of the predicted peptides (Majumder & Wu, 2010).

Dietary proteins through GIT loss most of their secondary, tertiary, and quaternary structures, but, some features of secondary structures such as disulfide bonds could not be lost easily. Thus, during *in-silico* BP mining, disulfide bonds should not be forgotten. Disregarding disulfide bond during generation of BP using *in-silico* method is the most important challenge for this method. The proteome-based method uses primary structure of a protein for generation of BP, while disulfide bond and other posttranslational modification do not consider in primary structure. This challenge could be solved easily, if the peptides that have AAs involved to a disulfide bond be linked together. Therefore, solving this challenge can yield BPs with new structures.

4.1.2 | Genome-based method

Each organism contains several types of proteins; therefore, it is virtually impossible to detect and enter all of these proteins in the proteome-based method. For that reason, the genome-based method was introduced to comprehensively predict the BPs found in each organism/food item. Anekthanakul, Hongsthong, Senachak, and Ruengjitchatchawalya (2018) designed an *in silico* digestion-based platform to assist the BPs prediction from a genome-wide database.

The common steps in the genome-based method are quite similar to the proteome-based except one, the first step, as protein selection. In the previous method, protein selection was manually done and based on the literature review, while in the genome-based method, the whole organism genome was entered as a template for whole proteome prediction. After the prediction of the whole proteome by *in-silico* genome translation, BPs discovery was made, as it was mentioned earlier (Anekthanakul et al., 2018).

In-silico approach for BPs characterization could help researchers to identify the importance of each digestive enzyme in the digestion process, when combined with *in-vivo* and *in-vitro* methods. Actually, coupling *in-silico* methods with *in-vitro* and *in-vivo* models have importance from two aspects as follows, as determining the importance role of each digestive enzyme in the digestion process. The studies used *in in-silico* methods are summarized in Table 2.

4.2 | *In-vitro* methods

In most studies, BPs have been characterized using in vitro models, which are based on the simulated digestion of food extracts by digesting with the gut simulant enzymes or microbial approach. There are several methods for *in vitro* digestion of food including static and dynamic methods. These methods were designed to simulate the physiological conditions of the upper GIT including the mouth, stomach, and small intestine (Minekus et al., 2014). Hence, due to the need for harmonization of the digestive conditions, the INFOGEST international network (www.cost-infogest.eu) was established by the

specialized experts from over 35 different countries. One of the main tasks of this network was establishing international consensus on the effective parameters for simulating *in vitro* static digestion for food (Brodkorb et al., 2019).

4.2.1 | Enzymatic methods

In this process, at an optimized temperature and pH, the protein materials were subjected to enzymatic hydrolysis. The enzymatic hydrolysis for producing the BPs has priority to microbial fermentation, due to the ease of scalability, predictability, and the short reaction time (Cian, Martínez-Augustin, & Drago, 2012). In this regard, to obtain the peptide sequences, various proteolytic enzymes were used to hydrolyze the protein (Mazorra-Manzano, Ramírez-Suarez, & Yada, 2018). However, to incorporate the enzyme into the isolated protein for hydrolysis, the enzyme activity temperature and optimal pH should be adjusted (Cao et al., 2017; Gibbs, Zougman, Masse, & Mulligan, 2004; Hernández-Ledesma, Quirós, Amigo, & Recio, 2007; Mohtar Wan, Hamid, Abd-Aziz, Muhamad & Saari, 2014).

Even though no special proteolytic enzymes are still recognized for the production of specific BPs in any type of food, subtilisin hydrolysis seems to be the best method for producing the low molecular weight (LMW) peptides, while some of them are bioactive (Khiari, Ndagijimana, & Betti, 2014). The proportion of enzyme and substrate is considered to be an important factor for obtaining a good level of hydrolysis. In this context, the peptides' orders and their biological activities can be correlated with the kind of enzyme used (Alu'datt et al., 2012; X. Wang, Dong, Li, Han, & Su, 2017), while the efficiency of the hydrolytic process may be affected by the produced protons, which were freed during the process of proteolysis resulting in the changes in the pH of the medium. Nevertheless, the fluctuation of pH can be modified by adding acid or alkali solutions; adjusting with alkali usually has some consequences in highly salted concentrations in the hydrolysates. Therefore, it can be recommended that the proteolysis should be performed in a suitable buffer (Wattanasiritham, Theerakulkait, Wickramasekara, Maier, & Stevens, 2016). The type of enzyme, temperature, and optimum time for enzyme activity are three important factors affecting the type of peptide produced from the protein hydrolysis (Lafarga & Hayes, 2017). After enzymatic hydrolysis, for separation of the supernatant, the mixture should be centrifuged that can separate the LMW peptides from the precipitates. However, the cross-flow membrane filtration, freeze-drying, desalting, and membrane ultrafiltration or column chromatography may help peptides to be recovered (X. Wang et al., 2017). Gel filtration, as a promising technique, can be used to quickly separate the LMW peptides, and furthermore, isolate them according to their sizes (Zhang et al., 2018). By solving the freeze-dried sample into a buffer (e.g., ammonium bicarbonate), hydrolyzed products of food protein can be prepared, following with sonication and cold centrifugation, while the supernatant was poured through an ultrafiltration membrane. To recover the maximum amounts of protein, the supernatant was added to ammonium carbonate as a buffer, and then once more, cold centrifugation was used to separate the supernatant. The precipitation of the solution was collected for profile peptides identification known as protein fraction. After the desalting of the solution, the peptides-dried samples were solved in acetonitrile and formic acid in deionized water (Y. Hou et al., 2014). In some studies, trifluoroacetic (TFA, 1% v/v) was used for sample preparation. Also, TFA was used as the mobile phase with methanol, acetonitrile, or water (Toll, Oberacher, Swart, & Huber, 2005). Then, reversed-phase high-performance liquid chromatography (RP-HPLC) was used for the separation of BPs, because of its efficiency, versatility, and automation abilities, while this technique also offers a suitable online coordination with mass spectrometry (MS). Several methods have been used to determine the BPs by HPLC such as liquid chromatography coupled with electrospray ionization quadrupole time of flight mass spectrometry (LC-ESI-QTOF-MS) (Babini, Tagliazucchi, Martini, Dei Più, & Gianotti, 2017), RP-HPLC-MALDI-TOF-Mass (Zhang et al., 2018), liquid chromatography mass spectrometry/mass spectrometry linear trap quadropole orbitrap (LC MS/MS LTQ-Orbitrap) (J. Chen et al., 2018), LC-ESI MS/MS (Brijesha & Aparna, 2017), and high-performance liquid chromatography-matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry/mass spectrometry (HPLC-MALDI-TOF MS/MS) (Srinivas & Prakash, 2010). Finally, sequences of peptides were clarified by the aid of protein search engines such as SwePep (http://www.swepep.org), BIOPEP-UWM (www.uwm.edu.pl/biochemia), EROP-Moscow (http://www.erop.inbi.ras.ru/), BioPD (http://biopd.bjmu.edu.cn), PepBank (http://pepbank.mgh.harvard.edu), MASCOT (http://www.matrixscience.com/search_intro.html), PEAKS (http://www.bioinfor.com), and PepArML (http://peparml.sourceforge.net) (Edwards, 2017; Fan et al., 2019; Liu & Pischetsrieder, 2017; Nardo, Añón, & Parisi, 2018).

4.2.2 | Microbial methods

In this method, bacteria or yeast were cultured on protein substances to produce the proteolytic enzymes, which cause protein hydrolysis, and furthermore result in the production of BPs (Chaves-López et al., 2014; Rafiq, Huma, Pasha, Shahid, & Xiao, 2017). At first, microorganisms were grown at the appropriate temperature to enter the exponential phase in a broth medium. Following this, the cells were collected, washed, and then suspended in sterile distilled water containing glucose, and were then used as an initiator to inoculate a sterilized protein substrate (Daliri et al., 2017). The bacterial

species, protein type, and fermentation time are among the important factors in determining the hydrolysis rate (Daliri et al., 2017). As an example, *Lb. helveticus* pose the capability of producing more peptides in fermented milk, due to its high extracellular proteinase activity (Griffiths & Tellez, 2013; Raveschot et al., 2018). Moreover, a combination of various bacteria or yeasts can accelerate the proteolysis of proteins. After the process of fermentation, the mixture was centrifuged, and then the supernatant liquid was recovered (Rizzello, Lorusso, et al., 2017). For producing smaller peptide sequences, the supernatant should be hydrolyzed using proteolytic enzymes. Alternatively, solvent extraction or other methods were used for the recovery of the LMW peptides in the supernatant. Accordingly, these were purified and their AA sequences can be analyzed using MS, and then identified using protein database search programs (Babini et al., 2017; Edwards, 2013; Liu & Pischetsrieder, 2017; Saraswathy & Ramalingam, 2011).

4.2.3 | Challenges in *in-vitro*-based methods of BPs production

It is notable that *in-vitro* characterized BPs do not necessarily show their activities *in-vivo* (Chakrabarti et al., 2018; Joel, Sutopo, Prajitno, Su, & Hsu, 2018), and this could be due to the digestion or unabsorbability of the peptides through GIT. Proteins are susceptible to large levels of hydrolysis in the GIT by the stomach, small intestinal, and brush border peptidases. As a result, many of the produced peptides cannot reach the absorption stage in the duodenum and jejunum (Chanphai & Tajmir-Riahi, 2017; Foltz et al., 2010; Keller & Layer, 2005). Therefore, despite the common use of enzymatic and microbial methods, these methods cannot be considered as a suitable method for simulation of digestion in the GIT (Bohn et al., 2018; Chakrabarti et al., 2018; Joel et al., 2018).

4.3 | *In-vivo* methods

Production of BPs by *in-vitro* methods is much easier than *in vivo*, because all peptides produced from *in vitro* method are originated from the food; while *in vivo*, in addition to exogenous peptides, endogenous peptides are also detected and their separation is very difficult. Accordingly, only a few studies have been conducted regarding the production of BPs by the aid of *in vivo* technique. The studies used *in-vivo* methods for BPs production and/or characterization that are demonstrated in Table 3.

4.3.1 | Aspiration of intestinal and stomach contents method

Based on the systematic search, AISC method was used to produce BPs, among some human studies (Boutrou et al., 2013; Boutrou, Henry, & Sanchez-Rivera, 2015; Chabance et al., 1998; Svedberg, de Haas, Leimenstoll, Paul, & Teschemacher, 1985). In this context, the appearance of digestion refractory peptides in the stomach and small intestine, using gastrointestinal intubation technique, was investigated.

In this method, after an overnight fasting, a tube was passed through the nasal cavity into the stomach and small intestine under the fluoroscopic control for sampling gastric and intestinal contents after meal ingestion. Gastric and duodenal contents were aspirated following any meal ingestion in the subjects who were all overnight fasting.

Then, the peptides were fractionated and sequenced using MS (Barbé et al., 2014; Chabance et al., 1998). The duodenum is the most important part of the intestine for digestion; therefore, in this method, duodenum content was mainly sampled. By passing half an hour from eating and at different times, samples were taken from the contents of the duodenum at 10 cm lower than pylorus and were then mixed with a protease inhibitor, homogenized, and frozen in −70 °C. Furthermore, they were analyzed by Liquid chromatography (LC)–MS/ MS and/or Matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF/TOF), to characterize the peptides throughout the digestion. Then, they were centrifuged, and supernatants were acidified by using TFA to pH = 2, diluted in guanidine hydrochloride 8 M 5:1 (v:v), and then filtered with the 30 kD membrane (Goldman et al., 2007). Guanidine hydrochloride is a strong chaotrope and considered as one of the strongest protein denaturants. Guanidine hydrochloride allows LMW peptides (0.9 to 5 KDa) to easily filtrate through the membrane. Then, the ultrafiltrate was desalted on a C_{18} cartridge before drying. Afterward, the dried ultrafiltrate was resuspended in 0.1% TFA/water and fractionated on RP_{-18} HPLC column. Each fraction was analyzed using MALDI-TOF/TOF and/or LC-MS/MS (An, Bekesova, Edwards, & Goldman, 2010; Ressom et al., 2008). To identify the peptides, all MS and/or MS/MS data were subjected to the protein database search engines, and for each identified peptide, a minimum score corresponding to a P-value < 0.05 was considered as a prerequisite for peptide validation corresponding to an average score (Boutrou et al., 2013; Chabance et al., 1998; Svedberg et al., 1985).

AISC method, while compared with *in-vitro*-based BPs characterization, is an invasive but more realistic one, while recognizing BPs in this technique does not actually mean to absorb them. Additionally, the brush border peptidases and serum peptidase cannot be identified in the aspiration method. In fact, the AISC method does not consider the absorbability aspect of BPs definition.

Although the AISC method cannot cover the absorbability aspect of BPs definition, it is very suitable for evaluating the interactions of the food matrix with BPs. Few studies have been performed regarding the effects of food matrix on the protein's digestion and the nature of formed peptides, and

the results have been sometimes inconsistent (Mennella, Visciano, Napolitano, Del Castillo, & Fogliano, 2006; A. Mohan, Udechukwu, Rajendran, & Udenigwe, 2015; Van Lancker, Adams, & De Kimpe, 2011). Also, the chemical structure of proteins can be accounted as an important factor to limit or modify the access of digestive enzymes to some cleavage sites. Also, peptides are highly reactive due to their nucleophilic structure. In this regard, during digestion, they are susceptible to altering the structure and reacting with the food matrix. The interactions can reduce the bioavailability of BPs, and can also change their biological activities. Moreover, the interaction of the food matrix with peptides may produce new compounds that may be bioactive or harmful (Udenigwe & Fogliano, 2017).

4.3.2 | Standard curve-fitting method

In this method, standard solutions of the *in-vitro*-characterized BPs (di or tripeptides) were purchased, and serial dilution of various standard concentrations was prepared. LC-MS/MS data were used for recognizing the concentrations of the solutions to draw the standard curve, plotting concentration on the *X*-axis, and performing the assay measurement on the *Y*-axis. Then, a similar assessment was done with serum samples of the unknown concentration. Serum samples were collected before and after the food ingestion (e.g., gelatin hydrolysate). To analyze the data, the researcher located the measurement on the *Y*-axis that corresponds to the assay measurement of the unidentified ingredients, and traced a line to intersect the standard curve. The attributed value on the *X*-axis was the level of a substance in the unknown sample (Ichikawa et al., 2010; K. Sato, 2017; Shigemura, Akaba, et al., 2011; Shigemura, Nakaba, et al., 2012; Shigemura, Suzuki, Kurokawa, Sato, & Sato, 2018). In this method, due to the specificity of the peptide that was evaluated, multiple reaction monitoring (MRM) can be used to increase the accuracy. Daughter ions have a high specificity for the detection of maternal fragments; therefore, in addition to *m*/*z*, daughter ions were targeted for identification (Freue & Borchers, 2012).

The SCF method measured the *in-vitro*-characterized peptides in the serum or plasma samples. After the food ingestion, different BPs including various molecular weights can be found in the blood; however, this method cannot characterize all of them. The SCF method was only used to identify the predetermined structures (Shigemura, Akaba, et al., 2011; Shigemura, Iwai, et al., 2009). In fact, this method can be regarded as a measurement method, not as a production or characterizing method.

4.3.3 | Postabsorption characterization

The blood contains a wide variety of endogenous peptides and proteins. Hormones and globulins made up a large part of the peptides and protein pool. Also, the peptides derived from degradation of endogenous proteins were also added into this pool. Therefore, blood contains various proteins in terms of molecular weight (D'Adamo et al., 2011; Wen & Jin, 2017). After the food ingestion, BPs were also added to the pool; thus, mixing the endogenous and exogenous peptides made it difficult to separate the BPs in the blood (Figure 2). In general, a two-step method is needed for characterizing the BPs in the blood as follows: (a) discriminating between endogenous and exogenous peptides and (b) characterizing the exogenous peptides.

Based on the findings, only one study was dealt with the characterization of BPs in the blood. Accordingly, Chabance et al. (1995) characterized Κ-casein derived BPs in the plasma of newborn after milk ingestion (Chabance et al., 1995). In this study, the infants' plasma peptide profile after milk ingestion was separated and then identified using HPLC, and the profile was compared to the plasma peptide profile of the adults of control under the fasting condition. The sequence of the unknown peaks was achieved by Edman degradation. The results of the unknown peaks characterization revealed that the Κ-casein-derived BPs with sequence IAIPPKKIQDK and MAIPPKKNQDK were absorbed into the blood after ingestion of milk in the infants. In this method, all aspects of BPs definition were considered; however, after this study, no attempt has been made to produce BPs by the use of this method. Chabance et al. (1995) performed a comprehensive research on the postabsorption production of BPs; however, the tools used by these researchers had no ability in characterizing heavier peptides (Chabance et al., 1995). In the 1990s, MS techniques were not well developed (Singhal, Kumar, Kanaujia, & Virdi, 2015). Nowadays, due to the remarkable progress in the mass spectroscopy field, this method has great potential for identifying the BPs.

Studies conducted over the last 20 years have tried to characterize the endogenous LMW peptides in the serum as diagnostic biomarkers, using MS and protein database search programs (Ai et al., 2015; An et al., 2010; Conrads et al., 2004; Conti et al., 2016; Goldman et al., 2007; Li, Zhang, Rosenzweig, Wang, & Chan, 2002; Martorella & Robbins, 2007; Ressom et al., 2008; Villanueva et al., 2004; Yin et al., 2018). Tirumalai et al. (2003) have designed an MS method to characterize the LMW peptides in the serum. In this method, centrifugal ultrafiltration of serum was performed to eliminate the bulky constituent proteins leading to the enrichment of the LMW proteins/ peptides. Then, the sample (LMW serum proteome) was digested using trypsin, and after that, it was fractionated by cation exchange chromatography, and was finally analyzed by microcapillary reversed-phase liquid chromatography coupled online with electrospray ionization tandem MS. Tandem MS spectra were searched against the human proteomic database implementation of SEQUEST operating on a Beowulf cluster, for identifying the

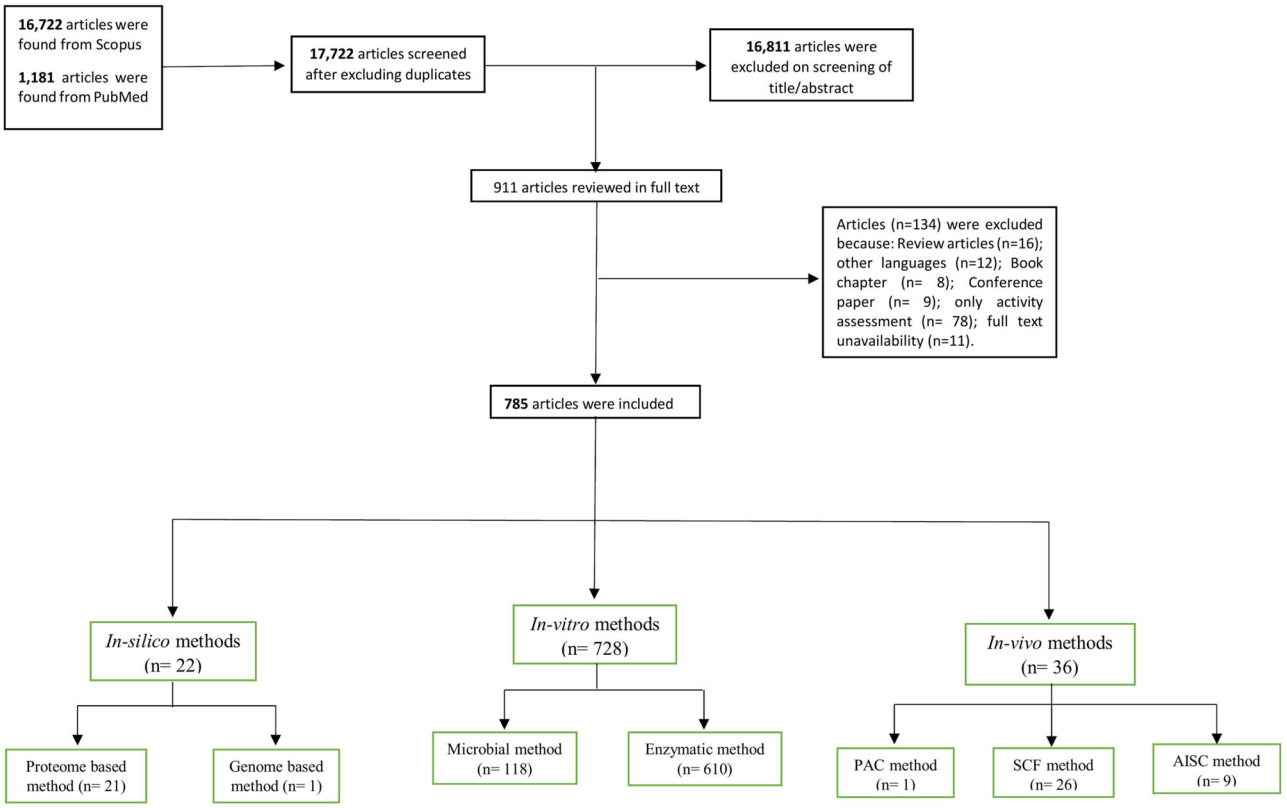

FIGURE 1 The study selection process

Abbreviations: AISC, aspiration of intestinal and stomach content method; SCF, standard curve fitting method; PAC, postabsorbtion characterization.

peptides sequences. In this study, analysis of the tandem mass spectra resulted in identifying over 340 human serum peptides; but not even a single peptide from serum albumin was observed (Tirumalai et al., 2003). Surprisingly, in a similar study, An et al. (2010) reported several LMW peptides as hepatocellular carcinoma (HCC) diagnostic biomarkers. Two groups of serum samples were enrolled in this study as follows: the serum samples of the patients with HCC and the healthy subjects (as control). The researchers characterized the LMW peptides in both samples using the method that Tirumalai et al. (2003) have designed. After that, the profile of the LMW peptides in the HCC patients was compared with the healthy controls. The study results served NGFKSHALQLNNRQI, DDPDAPLQPVTPLQLFEGRRN, VPPNNSNAAEDDLPTVELQGVVPR, and HVQPQPQPKPQVQLHVQSQT as diagnostic biomarkers of HCC (An et al., 2010).

The method used in An et al. (2010) study can be considered as a good model for BPs characterization in the blood (An et al., 2010). Determination of the LMW peptides profile under the fasting condition (as control), and the postingestion using MS, and intergroup comparison of both profiles can be considered as a comprehensive method for BPs characterization. Identifying the unknown MS peaks could be considered as the most important step in the method. After identifying the unknown MS peaks, the peaks should be converted to AA sequence *in-silico* using protein database search programs such as MASCOT, SEQUEST, and PepArML (Damodaran, Wood, Nagarajan, & Rabin, 2007; Edwards, 2013; Liu & Pischetsrieder, 2017; Saraswathy & Ramalingam, 2011) (Figure 3).

Recognizing the food components in the blood means that there are specific mechanisms for their absorption in the intestine. The recognition of BPs in the bloodstream indicated that they have a definite intestine absorption process (transcellular or paracellular) (Räder, Reichart, Weinmüller, & Kessler, 2018; Shen & Matsui, 2017, 2019; Vorherr, Lewis, Berghausen, Desrayaud, & Schaefer, 2018; Xu, Hong, Wu, & Yan, 2019), and this specific absorption way can help researchers in producing oral peptide drugs (Cammisotto et al., 2010; Villaseñor, Lampe, Schwaninger, & Collin, 2019). Bioinformatics simulation of BPs absorption is crucial to recover the peptides, which their absorption has already been proved (Hu & Smith, 2019; Marques et al., 2018). The BPs with high molecular weight are better for those proposed ways and mechanisms of absorption in the process of the newly produced peptide drugs (H. Fan, , Liao, & Wu, 2019; Lamberti, Cascone, Iannaccone, & Titomanlio, 2012; Minhas & Newstead, 2019); For example, when one BP including 30 AAs is identified in blood after food intake. Next, the identification of the absorption ways of this peptide *in-silico* and confirming it *in-vitro* and *in-vivo* will be big achievements in

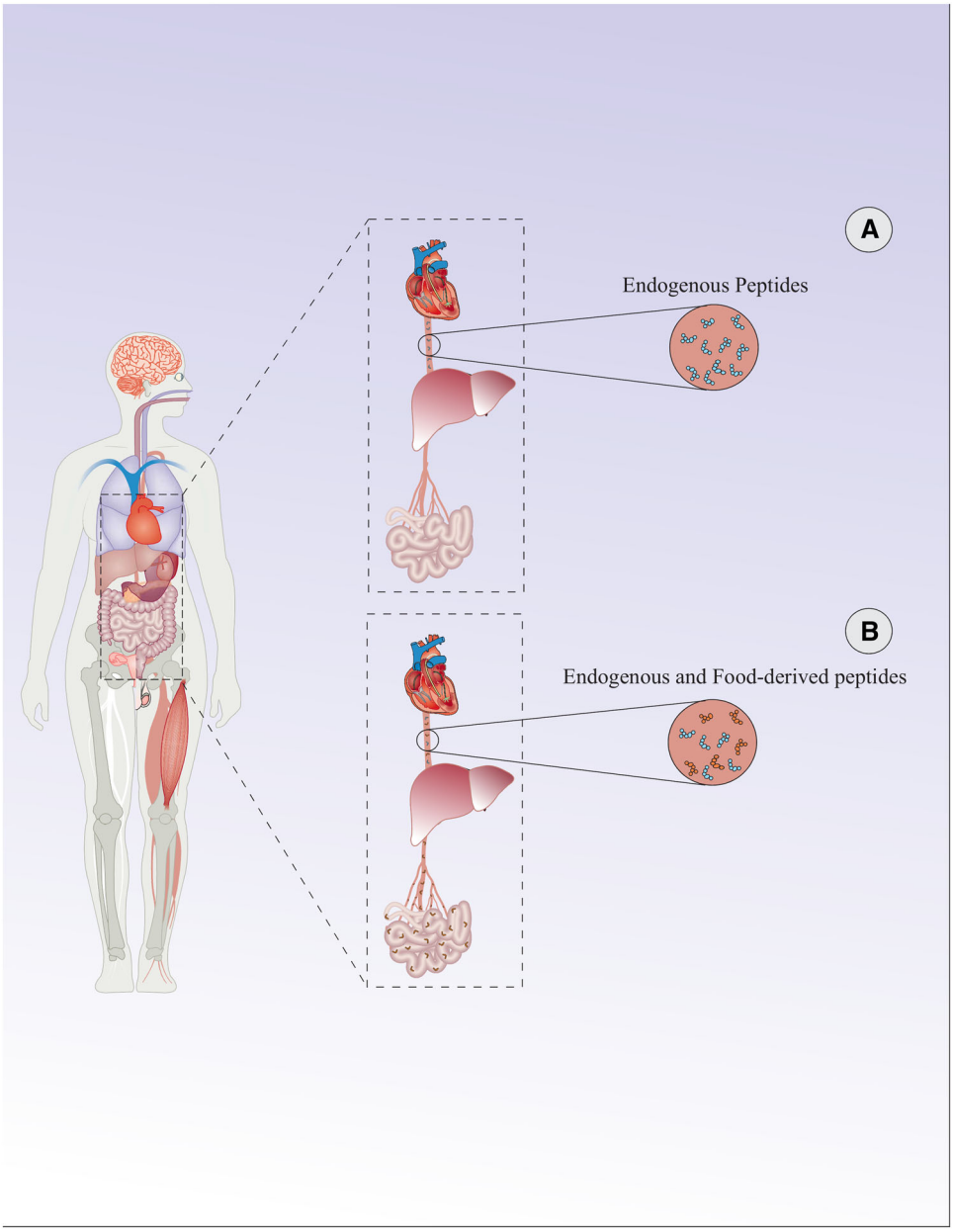

FIGURE 2 The blood peptide profile under fasting condition and in the postabsorptive phase. (a) The blood peptide profile under fasting condition. (b) The blood peptide profile in the postabsorptive phase. The blood contains various peptides in terms of molecular weight. After food ingestion, BPs are also added to the pool

the production of oral peptide drugs. Coupling the Glucagon with 29 AAs and insulin with 51 AAs with absorption mechanisms of the BPs will be great assistance in the drug delivery science and technology. For this purpose, the PAC of BPs should widely be developed.

4.4 | Challenges in BPs separation and characterization

BPs are usually separated using different chromatographic techniques. There are some problems and limitations for the separation and characterization of the BPs. Since BPs are often in very short sequences (two to six AAs); this is an important challenge for current proteomic techniques (Piovesana et al., 2018). Small peptides may not be suitable for the detection of MS, because they are often singly charged and it is very difficult to obtain the appropriate fragment (Nongonierma & FitzGerald, 2017). In addition, the classic database search method is not compatible in this case. Peptides of less than six AAs are not usually detectable in bioinformatics software with a high confidence, and the peptide is likely to be found in a whole range of protein sequences leading to a redundancy in MS/MS database search methods. The problem is more about peptides ACE-inhibitors, because they are

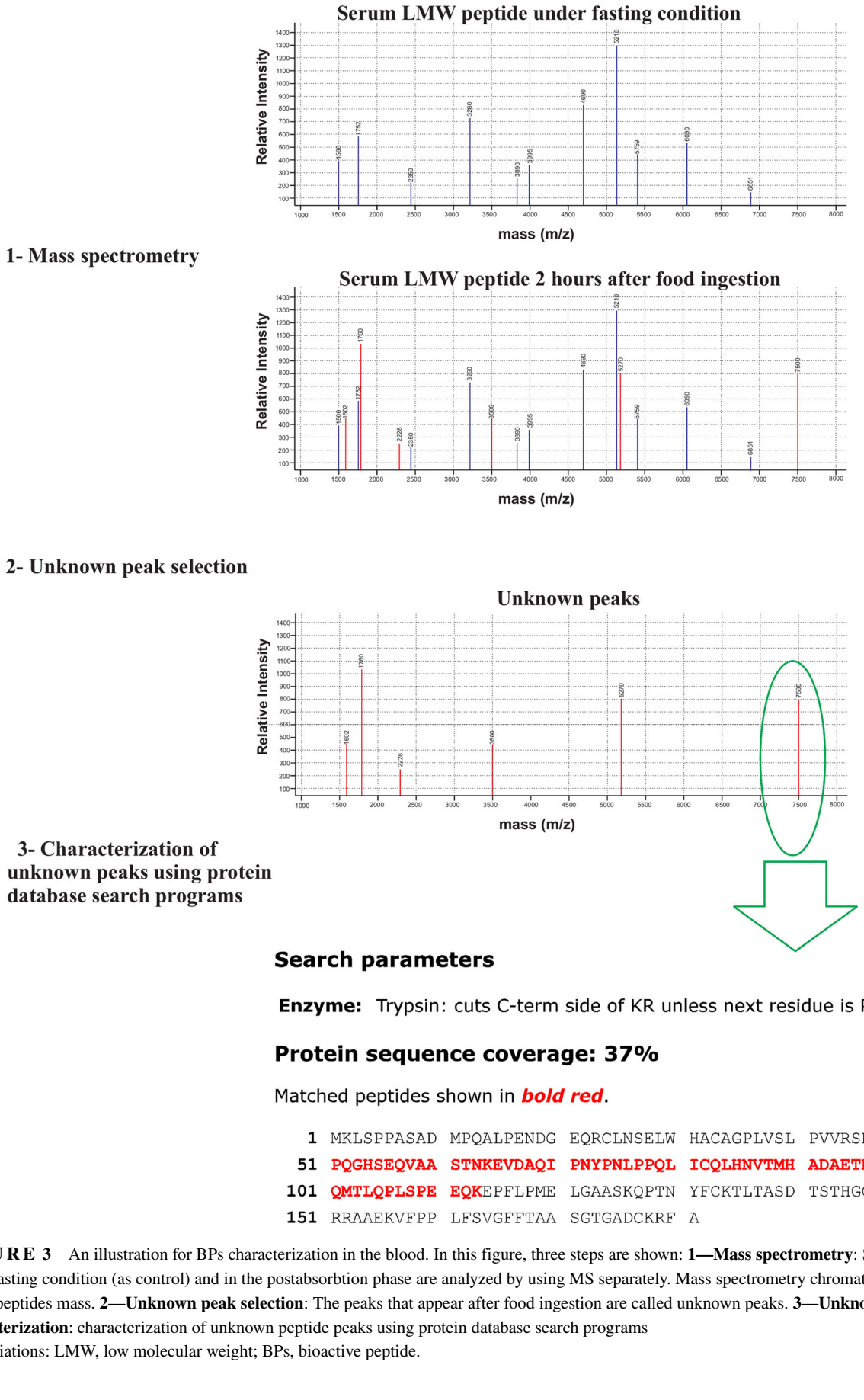

FIGURE 3 An illustration for BPs characterization in the blood. In this figure, three steps are shown: **1—Mass spectrometry**: Serum peptides under fasting condition (as control) and in the postabsorbtion phase are analyzed by using MS separately. Mass spectrometry chromatograms show serum peptides mass. **2—Unknown peak selection**: The peaks that appear after food ingestion are called unknown peaks. **3—Unknown peak characterization**: characterization of unknown peptide peaks using protein database search programs

Abbreviations: LMW, low molecular weight; BPs, bioactive peptide.

usually small peptides. It should be noted that small peptides require some specific separation techniques. The classical RP-HPLC and SEC methods used to separate BPs are not suitable for small peptides (Li-Chan, 2015).

Another issue in purification and fractionation of peptides is related to the scale. Peptide properties such as molecular mass, net charge, and hydrophobicity are important parameters that must be considered during the separation processes in industrial application (Rizzello, Tagliazucchi, et al., 2016). Solid-phase methods and techniques such as RP-HPLC are the most commonly used methods in the studies (laboratory scale) that allow the production of peptides up to 5 kg per year, but the problem is that they are expensive. Although peptides synthesized by chemical methods are cheaper, but they have no food application, and are also more commonly used as drugs and diagnostic targets (Lafarga & Hayes, 2017).

When MS is used to identify the peptides in complex matrices, protein databases are the most common methods for matching these spectra. This method is successful when the target protein database is complete, and the cleavage created by the digestive enzymes is completely clear. If the target protein database is not fully identified and the digestive enzyme is not site-specific, their identification process requires a longer time and the probability of identifying the related peptides is reducing (Armengaud et al., 2014; Mohayeji et al., 2014).

4.5 | BPs function and methods of BPs function assessment

4.5.1 | BPs function

In recent years, the major focus on BPs was on the more evidence-based wider use of them on specific health or medical benefits, including blood pressure lowering (BP), lipid lowering, anticancer, immune modulatory, antimicrobial, antioxidant, anti-inflammatory, and anticoagulation effects (Figure 4) (Cheng, Tu, Liu, Zhao, & Du, 2019; Park & Nam, 2015; Rutherfurd-Markwick, 2012; Yu, Wang, Zhang, & Fan, 2016). The types of enzymes and protein substrates are key factors in producing various BPs with dedicated functions. Recent publications reported the correlation of the peptides' functions with their AA sequences widely. Each AA in sequence fragments has its own properties in terms of hydrophilicity/hydrophobicity, size, polar, and charge (Qin et al., 2019).

Antihypertensive and antithrombotic peptides

To date, food-derived ACE inhibitors such as some BPs have attracted attention from a wide range of sources. According to previous reports, BPs in milk, soybean, animal muscle, wheat germ proteins, and fish proteins can be considered as an ACE inhibitors (Tu, Liu, et al., 2018; Tu, Wang, et al., 2018). Nasri et al. (2012) isolated and identified the four novel BPs sequences with high anticoagulant activity from goby muscle protein. Moreover, Khiari, Rico, Martin-Diana, & Barry-Ryan (2014) reported that bioactive gelatin peptides from mackerel (Scomber scombrus) skin hydrolysates can be able to inhibit platelet aggregation significantly and may be considered valuable due to its antithrombotic activity.

It has been known that ACE-inhibitory BPs are short in length, and their potency is strongly affected by C-terminal residue. Regarding dipeptides, hydrophobic side chains such as Phe, Trp, and Tyr were also preferred AA residues with bulky side chain (Pangestuti & Kim, 2017). Also, for tripeptides, the aromatic AAs are the most favorable residues for the C-terminal, for the middle position of them positively charged AAs preferred, and hydrophobic residues were favored for the N-terminus (Wu, Aluko, & Nakai, 2006). The bioinformatics analysis, quantitative structure-activity relationships (QSAR) model and molecular docking, revealed that the highest ACEI dipeptides had Trp (Lys-Trp and Val-Trp) at their C-terminus (Manzanares, Gandía, Garrigues, & Marcos, 2019). In this regard, among tripeptides, the highest potent ACE inhibitory properties of biological tripeptides correlated with having Pro (Met-Lys-Pro, Phe-Ala-Pro, Val-Ala-Pro, Ile-Pro-Pro, Val-Pro-Pro, and Lys-Arg-Pro) or Tyr (Ile-Val-Tyr) at the C-terminus. Regarding the regulation through the renin–angiotensin system (RAS) by BPs, it is known relevance of Trp in the sequences of these peptides. The most potent peptides (Ile-Trp, Leu-Trp, Val-Trp, and Ala-Trp) with renin inhibitory properties all possess Trp at the C-terminus and branched chain aliphatic residues, L or I, at the N-terminus (Udenigwe & Aluko, 2012). Arg-Tyr-Leu-Pro, Tyr-Thr-Ala-Trp-Val-Pro, and Tyr-Arg-Ala-Trp-Val-Leu were revealed as a novel inhibitory structure against both renin and ACE. For dual inhibition effect (renin and ACE inhibition), it was established that longer peptides such as tetrapeptides and pentapeptides are better candidates versus short peptides such as dipeptides (J.-H. Wang et al., 2013). Captopril was known as the first orally active ACE inhibitor that designed based on the results obtained from interaction among ACE, Ala-Pro, and Phe-Ala-Pro peptides (Cushman, Cheung, Sabo, & Ondetti, 1977).

Antidiabetic peptides

The most aimed target for antidiabetic BPs is the inhibition of metabolic enzymes involved in the regulation of serum glucose, including DPP-IV (EC: 3.4.14.5), α-amylase (EC: 3.2.1.1), and α-glucosidase (EC: 3.2.1.20). The aromatic AAs with polar side chain and proline at the N-terminus (such as Trp-Pro but also Tyr-Pro) were detected in the sequence alignment of different DPP-IV inhibitory BPs (Nongonierma & FitzGerald, 2014). DPP-IV is a ubiquitous enzyme that causes a loss in glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) activity through their cleave and inactivation. Regarding the inhibition of α-glucosidase, it has been made clear that the sequences

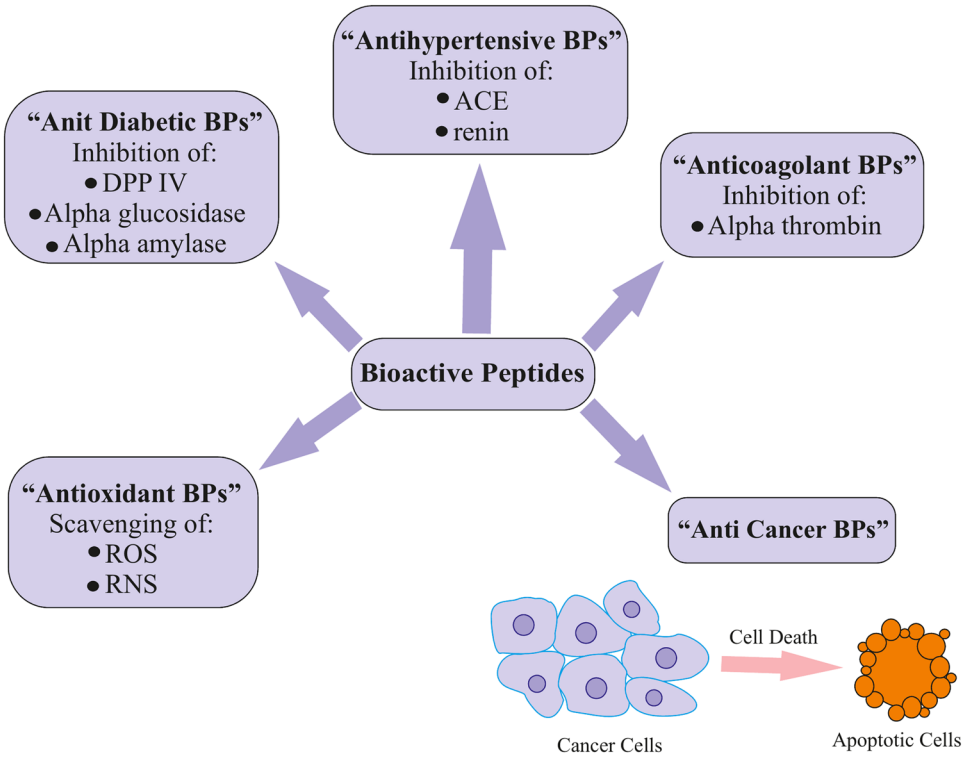

FIGURE 4 The biological functions of food-derived bioactive peptides

Abbreviations: BPs, bioactive peptides; ACE, angiotensin converting enzyme; DPP IV, dipeptidyl peptidase IV; ROS, reactive oxygen species; RNS, reactive nitrogen species.

including three to six AA residues with either Ser, The, Tyr, Lys, or Arg at the N-terminus and a Pro residue in the vicinity of the C-terminal with residues Met or Ala occupying the C-terminal position are necessary (M. A. Ibrahim, Bester, Neitz, & Gaspar, 2018). Ala-Lys-Ser-Pro-Leu-Phe, Gln-Thr-Pro-Phe, Phe-Glu-Glu-Leu-Asn, and Leu-Ser-Lys-Ser-Val-Leu were known as multifunctional antidiabetic BPs due to their potential inhibitory activities of DPP-IV, α-amylase, and α-glucosidase. These peptides inhibited the mentioned enzymes through hydrogen bonds, polar, and hydrophobic interactions (Mojica & de Mejía, 2016).

Antioxidant peptides

According to pioneering studies, exogenous antioxidants like some BPs are necessary to maintain the oxidative and antioxidative balance in biological systems (Ye et al., 2018). It is generally accepted that several AAs such as Tyr, Met, His, Lys, and Trp when incorporated into peptides may have potential health benefits with high activity and easy absorption without any side effects (Tu, Cheng, et al., 2018). Apart from this agreement, the lack of full knowledge regarding the structure activity of antioxidant peptides and their mechanism of actions as well the lack of a standardized methodology for evaluating the *in vitro* antioxidant activity for comparison various results from different laboratories are major hampers in this realm (Zou, He, Li, Tang, & Xia, 2016).

Anticancer peptides

A growing body of biological peptides from natural animal sources is being considered as novel drug candidates for cancer therapeutic applications. Although some major mechanisms for the anticancer effects of the BPs have been proposed, the precise mechanism of their anticancer effects is still controversial. In general, the anticancer effect of BPs may be associated with some membrane or nonmembrane mechanisms (X. Wang et al., 2017; Zhou et al., 2017). The plasma membrane contains two phospholipid layers as a selective barrier that are essential to cell survival and function. Several studies have shown that antimicrobial peptides destroy the function of cancer cells by disrupting the cell membrane (Lohner & Hilpert, 2016; L. Wang, Dong, Li, Han, & Su, 2017). Specifically, the peptides target is negatively charged membrane components including phosphatidylserin, sialic acid, and heparan sulfate (Riedl et al., 2011). In fact, the BPs distinguish between cancer and noncancer cells by targeting the negatively charged lipid layer on the outer leaflet of the cancer cell membrane (Riedl et al., 2015). The effect of each peptide on the membrane activity of the cancer cells depends on the characteristics of the BP and the target membrane antibody. Membrane dysfunction occurs in a variety of ways including lipid pore formation, membrane layer thinning, membrane dissolution, or lipid–peptide domain formation (Rashid, Veleba, & Kline, 2016). In addition to

disrupting the plasma membrane, some anticancer peptides induce apoptosis through the mitochondrial pathway (Whelan et al., 2012). Therefore, the apoptosis induced by mitochondrial membrane dysfunction plays an important role in the treatment of cancer (Mulder, Lima, Miranda, Dias, & Franco, 2013). Therapeutic BPs have several significant advantages such as small size, minimal drug-drug interaction, high ability to penetrate the cell membranes, easy synthesis, and minimum toxic side effects. However, metabolic instability, poor oral bioavailability, and rapid clearance are some disadvantages of therapeutic BPs in this regard (Marqus, Pirogova, & Piva, 2017).

Basic studies and clinical findings

Many *in-vivo* (M. Sato et al., 2002; Yasufuku et al., 2001) and randomized clinical trials (RCTs) (Benito-Ruiz et al., 2009; Bruyere et al., 2012; Trc & Bohmova, 2011) evaluated the oral administration of BPs and BP-contained proteins on health outcomes. Sato et al. in an *in-vitro* study characterized Wakame-derived BPs with ACE inhibitory function. The *in-vitro* part of the Sato study represented Val-Tyr, Ile-Tyr, Ala-Trp, Phe-Tyr, Val-Trp, Ile-Trp, and Leu-Trp as antihypertensive BPs. In the *in-vivo* part, the researchers evaluated the effect of oral administration of the peptides with 1 mg/kg on blood pressure in spontaneously hypertensive rats (SHRs). The *in-vivo* results revealed that Val-Tyr, Ile-Tyr, Phe-Tyr, and Ile-Trp significantly decrease systolic blood pressure in the rats (M. Sato et al., 2002). In a similar study, Nii et al. reported antihypertensive effects of shrimp-derived BPs, Val-Trp-Tyr-His-Thr, and Val-Trp in SHR (Nii, Fukuta, Yoshimoto, Sakai, & Ogawa, 2008). The mentioned studies used purified BPs for application in *in-vivo*, while some experimental studies used BP-contained proteins. Yasufuku et al. (2001) examined the effect of oral administration of collagen V on lung allograft rejection and found that the treatment reduces the allograft rejection by induction of oral tolerance. In a similar study, Roldán et al. revealed that oral administration of glycomacropeptide, a milk-derived BP with 64 residues, prevents immune response in asthma and progression of the disease (Roldan, Jimenez, Cervantes-Garcia, Marin, & Salinas, 2016). Both of the studies used BP-contained proteins, and oral tolerance induction is the primary outcome for their results. Many experimental studies similar to Roldán et al. (2016) and Yasufuku et al. (2001) were performed and within their framework concluded that oral tolerance is the main mechanism for the therapeutic effects of BP-contained proteins (Chen, Bao, Wang, Xie, & Wu, 2012; Cui et al., 2017; Moronta, Smaldini, Fossati, Añon, & Docena, 2016; Nishikimi et al., 2018; Xi et al., 2009).

From the studies used BP-contained proteins, two important points should be considered: (a) the BPs should not be absorbed and entered into the circulation to exert their beneficial effects necessarily. BPs induce oral tolerance, an anti-inflammatory process, in the gut especially before digestion and when they are encrypted in their parent proteins. The gut immune system continuously samples the gut content and almost always tolerance versus dietary proteins happen (Pabst & Mowat, 2012) and (b) immune tolerance induction is very beneficial for prevention and treatment of cardiovascular disease (Figure 5) (Wigren et al., 2012), but Barth et al. (2015) in a nested case-cohort study revealed that factors related to immune tolerance increase risk of some solid cancers.

Poor chemical and physical stability, short plasma half-life, and low and variable bioavailability are crucial problems of bioactive compounds for their application as nutraceuticals or functional foods. The stability of BPs through GITcould be evaluated by finding trypsin, chymotrypsin, and pepsin cutting position (Manzanares et al., 2019). Commercially synthesis of BPs for management and treatment of hypertension, diabetes, and other chronic disease may not be cost/benefit effective. Nevertheless, structure-activity studies are preferable for designing the important drugs based on the results obtained from the BPs (Cushman et al., 1977).

4.5.2 | Methods of BP function assessment

In this systematic review, we comprehensively assessed the methods of BP generation. BPs have wide and different functions, and methods of function assessment for BPs are all different from methods of BP generation. In brief, function assessment for BPs could be performed by different methods including: (a) prediction of BPs function by QSAR models, (b) enzyme inhibitory function assessment, (c) antimicrobial and antioxidant capacity assessment, and (d) anticancer activity assessment.

In biological and chemical sciences, QSAR models are used as classification models. First of all, in these models, a supposed association between two factors in chemicals data set including biological activity and chemical structures is summarized. On the other hand, about new chemical substances, QSAR models could predict activities. In fact, QSAR models let us predict the function of a BP based on similarity with prestructured library. Although *in-silico* prediction of BPs function is cost-effective and time-saving, the role achieved *in-silico* should be confirmed *in-vitro* and *in-vivo* (Deng et al., 2019).

The enzyme inhibitory function of BPs is an *in-vitro* method and is based on the ability of a given enzyme to hydrolyze the internally quenched fluorescent substrate, for instance, o-aminobenzoylglycyl-p-nitro-l-phenylalanyl-L-proline for ACE (Sentandreu & Toldra, 2006) and Gly-Pro p-nitroanilide for DPP-IV (Beckenkamp et al., 2015), in the presence of the BP. After incubation of a BP with a given enzyme in the presence of the internally quenched fluorescent substrate, generation of fluorescence is measured and enzyme inhibition is expressed as percentage (H. R. Ibrahim,

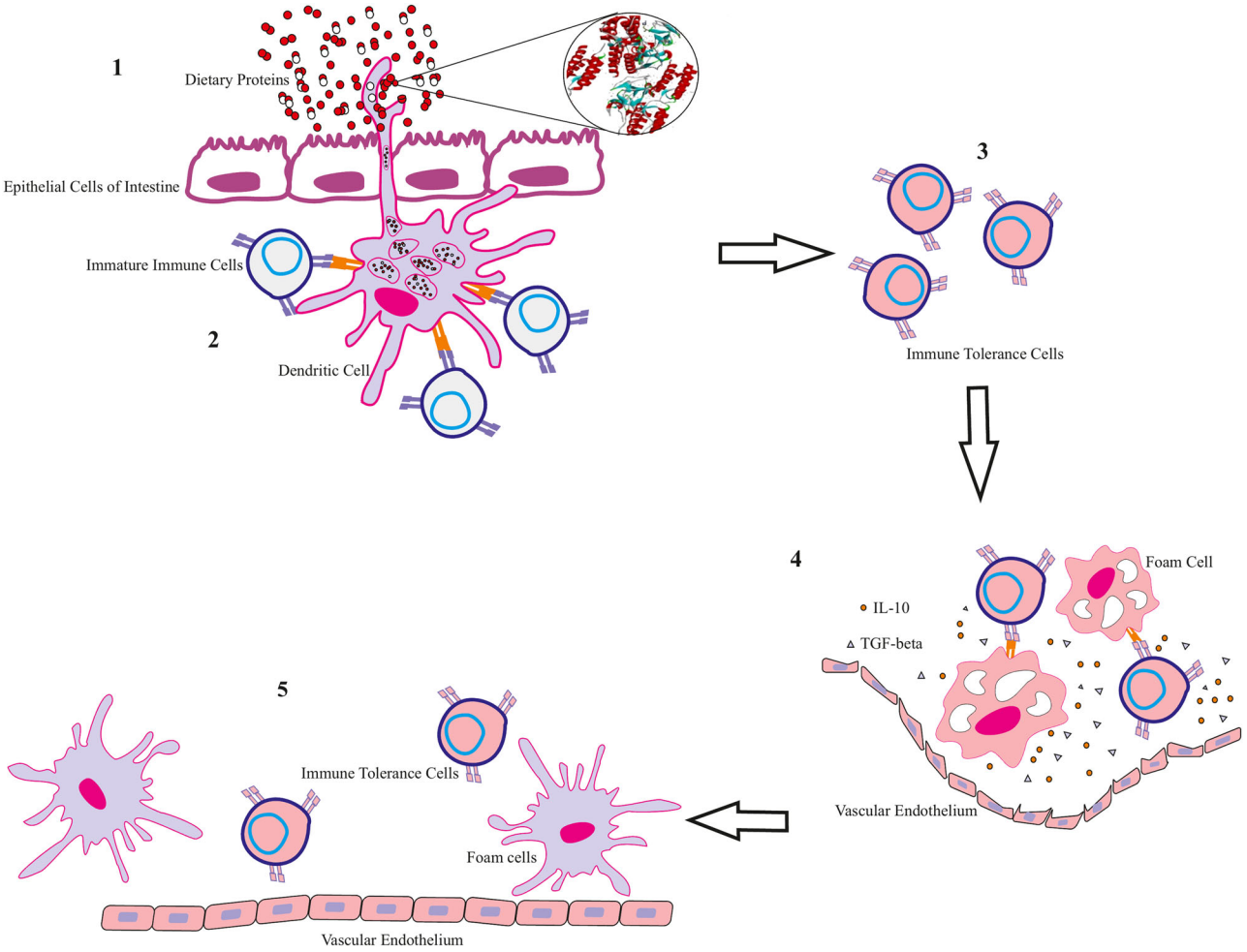

FIGURE 5 The effects of BP-contained proteins on cardiovascular health. 1—Sampling of dietary protein content of the intestine by dendritic cells located in the gut. 2—Dendritic cells that activated by BP-contained proteins interact with immature immune cells; 3—Immature immune cells after interaction with activated dendritic cells turn into a kind of mature immune cells called immune tolerance cells or regulatory T cells. 4—Migration of immune tolerance cells to atherosclerotic plaques (cardiovascular endothelium) and interaction with inflammatory cells (foam cells) caused cardiovascular disease. 5— Suppression of foam cells function by immune tolerance cells and improvement of endothelium
Abbreviations: IL-10, interleukin 10; TGF-beta, transforming growth factor-beta.

Ahmed, & Miyata, 2017). On the other hand, By means of disc diffusion assay, BPs antibacterial activity is examined. In the nutrient broth, activation of indicator microorganisms is happen. On the indicator strain that is prepared freshly, sample aliquots are spotted. Then, at the optimum temperature, incubation of plates is done. Diameters of zone in which growth is inhibited are measured subsequently. Then the "inhibition zones" are reported. In this regard, sterilized water and streptomycin are negative and positive controls, respectively (N. M. Mohan et al., 2019; Offret, Fliss, Bazinet, Marette, & Beaulieu, 2019). In a different method, antioxidant capacity of BPs is assessed. Method of 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic (ABTS) assay is a common used assay in assessment of antioxidant capacity. Basis of this method is as follows: Reduction in ABTS acid cations through the antioxidant peptides addition leads to decolorization of reagent. This is spectrophotometrically measureable and depends on some factors such as reaction time, concentration, and type of antioxidant (Aloglu & Oner, 2011; Ayala-Niño et al., 2019). Other methods for measuring of antioxidant activity are included oxygen radical absorbance capacity, total radical trapping antioxidant potential, 2,2 diphenyl-1 picryl-hydrazyl, ferric reducing antioxidant power, enhanced chemiluminesence, and electron spin resonance (Power, Jakeman, & FitzGerald, 2013).

For anticancer activity assessment, cancer cell line should be exposed to BPs and after exposure, cell growth and apoptosis should be evaluated by proliferation assays and flow cytometry, and if a BP insert apoptosis and reduce cell growth then, by evaluation of intracellular signals using real-time polymerase chain reaction (PCR) and western blot, the exact mechanism could be clarified (Ma et al., 2015; Su, Shi, Yan, Xi, & Su, 2015). For hypolipidemic function assessment, a protocol like this method should be performed, but the

difference is that BPs are mostly exposed to HepG2 cell line, human liver cancer cell line (Inoue et al., 2011).

There are many methods for assessment of BPs function, and here, we described some more common methods, briefly; therefore, "the methods of BP function assessment" could be a good topic for preparation of a systematic review. A most important point about function assessment is that any function obtained *in-vitro* or *in-silico* should be confirmed *in-vivo*.

4.6 | Nonfood derived BPs

In the current systematic review, however, the food-derived BPs were discussed, there are many BPs with nonfood sources that may originate from nonnutritional origins even dangerous sources such as the venom or may obtained by the aid of some bioinformatics assays. In this regard, the anticoagulant BPs, nonfood derived BPs, have been investigated more than food-derived BPs, while the most important source of nonfood derived BPs is the venom of different animals such as snake, frog, and bee (Pennington, Czerwinski, & Norton, 2018). Venoms from different animals such as snake in low doses have been used by some traditional remedies for treating different disease such as cancers, gastrointestinal disease, and arthritis (Casewell et al., 2014; Pennington et al., 2018). While the venom is a heterogeneous mixture of organic molecules, peptides, enzymes, and inorganic salts, the venom-derived BPs should be purified as single peptides, to be used as the drugs.

As mentioned above, most venoms are a mixture of peptides, proteins, enzymes, organic, and inorganic materials. Due to technology progresses over the past decades, it became easier to separate and purified venoms, especially as peptides and proteins. For the first time in 1980s, classical chromatography was used to purify the venom content and subsequently sequenced the fractions using the Edman degradation or mass-spectroscopic techniques. Due to the presence of disulfide bonds, many of nonfood derived BPs are highly structured (Petras et al., 2019). With advancement in nuclear magnetic resonance instrumentation, three-dimensional structures of the venom components have been obtained (Ramirez, Shekhtman, & Pande, 2018). Unlike food-derived BPs, for separation, identification, and characterization of nonfood derived BPs, no digestion is needed (Mourão & Schwartz, 2013).

Previously, intracerebroventricular injection of nonfood derived BPs into mice was used for the assessment of peptide's function (El Hidan et al., 2016). Also, heterologous expression of specific ion channels in eukaryotic cells by microinjection of cDNA has been applied to determine the ion channel blocking function of nonfood-derived BPs (Pennington et al., 2018). Another method for function assessment of nonfood-derived BPs is fluorometric substrate-based assays that are used for identifying inhibitors of biological fluid enzymes, especially blood-clotting enzymes (van der Wel et al., 2015). Other assays, such as the surface plasmon resonance (Choudhury, Konwar, Kaur, Doley, & Mondal, 2018) and fluorescence imaging plate reader (Jin et al., 2019), have significantly accelerated BPs identification.

Although the nonfood-derived BPs have higher affinity to their target and effectively exert their biological function in comparison with food derived BPs, the sources of nonfood derived BPs are rare and very expensive (Estevao-Costa, Sanz-Soler, Johanningmeier, & Eble, 2018). Also, the nonfood-derived BPs could not be administered orally, due to instability of their structure through GIT. As mentioned about food-derived BPs, identification of biologically active fragments is performed after *in-silico*, *in-vitro*, or *in-vivo* digestion, while, for nonfood-derived peptides, digestion only destructs the active fragments (Mourão & Schwartz, 2013). It should be noted that in heavy protein sequencing, digestion is used to reduce protein size to allow for more accurate sequencing (Lomazi, Nishiduka, Silva, & Tashima, 2018).

4.7 | BP synthesis

The methods described in the current investigation are strictly for BP identification, once a peptide sequence with desirable bioactive properties has been identified, and then, it would be prepared through a synthetic technique. The techniques for peptide/protein synthesis could be divided into two subgroups including chemical and chemoselective Ligation methods (W. Hou, Zhang, & Liu, 2017). Chemoselective ligation techniques are usually used for protein synthesis (Tiefenbrunn & Dawson, 2010), while chemical methods can be used for the synthesis of BPs (Zhu, He, & Hou, 2019).

4.7.1 | Chemical methods for BP synthesis

There are two main chemical methods for producing peptides in industrial/laboratory scales including solution phase synthesis (SPS) and solid-phase peptide synthesis (SPPS) (Jaradat, 2018). In the classical SPS, the method is based on single AA pairing in solution, while for producing long peptide, the fragment condensation method is used. First, short segments of the identified peptide are synthesized. Then, these fragments are paired together and a long peptide is formed. SPS method can be nominated as in a simple and inexpensive way, while purification of intermediate products can be archived at the high level of purity. On the other hand, the disadvantage of the SPS technique is a long reaction time (Chandrudu, Simerska, & Toth, 2013).

In terms of supporting for growing peptide, the resin is used as an anchor within the SPPS method. In this case, attachment of C-terminus of the first AA and the resin happens. Demonstrating both alpha-amino group (as antipolymerization agent) and some groups on the reactive side chain, which are temporary protecting the groups, is a distinctive characteristic of the first AA. By adding subsequent AA, the protection group

of the first AA is removed and the resin is washed before the adding the subsequent AA to the chain. This process is repeating until the completion of peptide sequence, and then, the desired peptide is dissociated from the resin (Chandrudu et al., 2013); For instance, two protecting groups including tert-butyloxycarbonyl (Boc) and fluorenylmethyloxycarbonyl (Fmoc) are often used (Behrendt, White, & Offer, 2016; Raz et al., 2016). Development of microwave-assisted SPPS leads to a notable improvement in SPPS synthesis, especially in long peptides synthesizing. However, the high efficacy in peptide production and low degrees of racemization can be achieved by the aid of the microwave radiance (Vanier, 2013). Also, this technique has some limitations including the expense of its equipment besides the cost of resin and reagents used in this technique (Chandrudu et al., 2013).

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

Three different models of BPs production were assessed by the aid of a systematic review. The *in-vitro* model, including microbial and enzymatic methods and the *in-vivo* model, including PAC, AISC, and SCF methods, have been used for BPs characterization. Recently, *in-silico* methods have also been suggested to predict BPs. So far, characterization of BPs has been mainly investigated by enzymatic and microbial *in-vitro* methods; however, despite the common use of the methods, they cannot be considered as suitable methods for simulation of digestion in GIT. Hence, the *in-vivo* and *in-silico* methods of BPs production were developed to solve this issue. Although the *in-vivo*-based methods have their disadvantages, with the further advancements in the field of mass spectroscopy and bioinformatics, the *in-vivo* methods gained the opportunity to be expanded.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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AUTHOR CONTRIBUTIONS

Meisam Barati, Fardin Javanmardi, Dr. Seyed Mohamad Hosein Mosavi Jazayeri, Masoumeh Jabbari, Jamal Rahmani, Farzaneh Barati, and Hamid Nickho prepared and wrote the manuscript. Professor Sayed Hossein Davoodi, Dr. Neda Roshanravan, and Dr. Amin Mousavi Khaneghah supervised and provided guidance in manuscript finalization.

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REFERENCES

Admassu, H., Gasmalla, M. A. A., Yang, R., & Zhao, W. (2018). Bioactive peptides derived from seaweed protein and their health benefits: Antihypertensive, antioxidant, and antidiabetic properties. *Journal of Food Science*, *83*(1), 6–16. https://doi.org/10.1111/1750-3841.14011

Ai, T., Chen, F., Zhou, S., Zhang, J., Zheng, H., Zhou, Y., … Lin, J. (2015). Magnetic bead-based serum peptidome profiling in patients with gestational diabetes mellitus. *Biomed Research International*, *2015*, 586309. https://doi.org/10.1155/2015/586309

Aloglu, H. S., & Oner, Z. (2011). Determination of antioxidant activity of bioactive peptide fractions obtained from yogurt. *Journal of Dairy Science*, *94*(11), 5305–5314. https://doi.org/10.3168/jds.2011-4285

Alu'datt, M. H., Ereifej, K., Abu-Zaiton, A., Alrababah, M., Almajwal, A., Rababah, T., & Yang, W. (2012). Anti-oxidant, anti-diabetic, and anti-hypertensive effects of extracted phenolics and hydrolyzed peptides from barley protein fractions. *International Journal of Food Properties*, *15*(4), 781–795. https://doi.org/10.1080/10942912.2010.503357

Aluko, R. E. (2015). Antihypertensive peptides from food proteins. *Annual Review of Food Science and Technology*, *6*, 235–262. https://doi.org/10.1146/annurev-food-022814-015520

An, Y., Bekesova, S., Edwards, N., & Goldman, R. (2010). Peptides in low molecular weight fraction of serum associated with hepatocellular carcinoma. *Disease Markers*, *29*(1), 11–20. https://doi.org/10.3233/dma-2010-0721

Anekthanakul, K., Hongsthong, A., Senachak, J., & Ruengjitchatchawalya, M. (2018). SpirPep: An in silico digestion-based platform to assist bioactive peptides discovery from a genome-wide database. *BMC Bioinformatics*, *19*(1), 149–149. https://doi.org/10.1186/s12859-018-2143-0

Armengaud, J., Trapp, J., Pible, O., Geffard, O., Chaumot, A., & Hartmann, E. M. (2014). Non-model organisms, a species endangered by proteogenomics. *Journal of Proteomics*, *105*, 5–18.

Asai, T., Takahashi, A., Ito, K., Uetake, T., Matsumura, Y., Ikeda, K., … Sato, K. (2019). Amount of collagen in the meat contained in Japanese daily dishes and the collagen peptide content in human blood after ingestion of cooked fish meat. *Journal of Agricultural*

and Food Chemistry, *67*(10), 2831–2838. https://doi.org/10.1021/acs.jafc.8b06896

Atamaleki, A., Yazdanbakhsh, A., Fakhri, Y., Mahdipour, F., Khodakarim, S., & Khaneghah, A. M. (2019). The concentration of potentially toxic elements (PTEs) in the onion and tomato irrigated by wastewater: A systematic review; meta-analysis and health risk assessment. *Food Research International*, *125*, 108518.

Ayala-Niño, A., Rodríguez-Serrano, G. M., González-Olivares, L. G., Contreras-López, E., Regal-López, P., & Cepeda-Saez, A. (2019). Sequence identification of bioactive peptides from amaranth seed proteins (Amaranthus hypochondriacus spp.). *Molecules (Basel, Switzerland)*, *24*(17), 3033. https://doi.org/10.3390/molecules24173033

Babini, E., Tagliazucchi, D., Martini, S., Dei Piu, L., & Gianotti, A. (2017). LC-ESI-QTOF-MS identification of novel antioxidant peptides obtained by enzymatic and microbial hydrolysis of vegetable proteins. *Food Chemistry*, *228*, 186–196. https://doi.org/10.1016/j.foodchem.2017.01.143

Barati, M., Yousefi, M., Ebrahimi-Mameghani, M., Mohammadi, H., Brazvan, B., Nickho, H., … Mohammadi, M. (2017). Oryzatensin-stimulated PBMCs increase cancer progression *in-vitro*. *Iranian Journal of Allergy Asthma and Immunology*, *16*(2), 120–126.

Barbé, F., Le Feunteun, S., Rémond, D., Ménard, O., Jardin, J., Henry, G., … Dupont, D. (2014). Tracking the in vivo release of bioactive peptides in the gut during digestion: Mass spectrometry peptidomic characterization of effluents collected in the gut of dairy matrix fed mini-pigs. *Food Research International*, *63*, 147–156. https://doi.org/10.1016/j.foodres.2014.02.015

Barth, S. D., Schulze, J. J., Kuhn, T., Raschke, E., Husing, A., Johnson, T., … Olek, S. (2015). Treg-mediated immune tolerance and the risk of solid cancers: Findings from EPIC-Heidelberg. *Journal of the National Cancer Institute*, *107*(11), djv224. https://doi.org/10.1093/jnci/djv224

Bauchart, C., Morzel, M., Chambon, C., Mirand, P. P., Reynes, C., Buffiere, C., & Remond, D. (2007). Peptides reproducibly released by in vivo digestion of beef meat and trout flesh in pigs. *Br J Nutr*, *98*(6), 1187–1195. https://doi.org/10.1017/s0007114507761810

Beckenkamp, A., Willig, J. B., Santana, D. B., Nascimento, J., Paccez, J. D., Zerbini, L. F., … Buffon, A. (2015). Differential expression and enzymatic activity of DPPIV/CD26 affects migration ability of cervical carcinoma cells. *PLoS One*, *10*(7), e0134305. https://doi.org/10.1371/journal.pone.0134305

Behrendt, R., White, P., & Offer, J. (2016). Advances in Fmoc solid-phase peptide synthesis. *Journal of Peptide Science*, *22*(1), 4–27. https://doi.org/10.1002/psc.2836

Benito-Ruiz, P., Camacho-Zambrano, M. M., Carrillo-Arcentales, J. N., Mestanza-Peralta, M. A., Vallejo-Flores, C. A., Vargas-Lopez, S. V., … Zurita-Gavilanes, L. A. (2009). A randomized controlled trial on the efficacy and safety of a food ingredient, collagen hydrolysate, for improving joint comfort. *International Journal of Food Sciences and Nutrition*, *60*(Suppl 2), 99–113. https://doi.org/10.1080/09637480802498820

Bhat, Z. F., Kumar, S., & Bhat, H. F. (2017). Antihypertensive peptides of animal origin: A review. *Critical Reviews in Food Science and Nutrition*, *57*(3), 566–578.

Blanco-Míguez, A., Fdez-Riverola, F., Lourenço, A., & Sánchez, B. (2019). In silico prediction reveals the existence of potential bioactive neuropeptides produced by the human gut microbiota. *Food Research International*, *119*, 221–226. :https://doi.org/10.1016/j.foodres.2019.01.069

Blanco-Míguez, A., Gutiérrez-Jácome, A., Fdez-Riverola, F., Lourenço, A., & Sánchez, B. (2017). MAHMI database: A comprehensive MetaHit-based resource for the study of the mechanism of action of the human microbiota. *Database: The Journal of Biological Databases and Curation*, *2017*, baw157. https://doi.org/10.1093/database/baw157

Bohn, T., Carriere, F., Day, L., Deglaire, A., Egger, L., Freitas, D., … Menard, O. (2018). Correlation between *in vitro* and in vivo data on food digestion. What can we predict with static *in vitro* digestion models? *Critical Reviews in Food Science and Nutrition*, *58*(13), 2239–2261.

Boutrou, R., Gaudichon, C., Dupont, D., Jardin, J., Airinei, G., Marsset-Baglieri, A., … Leonil, J. (2013). Sequential release of milk protein-derived bioactive peptides in the jejunum in healthy humans. *American Journal of Clinical Nutrition*, *97*(6), 1314–1323. https://doi.org/10.3945/ajcn.112.055202

Boutrou, R., Henry, G., & Sanchez-Rivera, L. (2015). On the trail of milk bioactive peptides in human and animal intestinal tracts during digestion: A review. *Dairy Science & Technology*, *95*(6), 815–829. https://doi.org/10.1007/s13594-015-0210-0

Bouzerzour, K., Morgan, F., Cuinet, I., Bonhomme, C., Jardin, J., Le Huërou-Luron, I., & Dupont, D. (2012). In vivo digestion of infant formula in piglets: protein digestion kinetics and release of bioactive peptides. *British Journal of Nutrition*, *108*(12), 2105–2114. https://doi.org/10.1017/S000711451200027X

Boyd, S. E., Garcia de la Banda, M., Pike, R. N., Whisstock, J. C., & Rudy, G. B. (2004). PoPS: A computational tool for modeling and predicting protease specificity. *Proceedings of IEEE Computational Systems Bioinformatics Conference*, 372–381. https://doi.org/10.1109/csb.2004.1332450

Brijesha, N., & Aparna, H. (2017). Comprehensive characterization of bioactive peptides from Buffalo (Bubalus bubalis) colostrum and milk fat globule membrane proteins. *Food Research International*, *97*, 95–103.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., … Carrière, F. (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nature Protocols*, *14*(4), 991–1014.

Bruyere, O., Zegels, B., Leonori, L., Rabenda, V., Janssen, A., Bourges, C., & Reginster, J. Y. (2012). Effect of collagen hydrolysate in articular pain: A 6-month randomized, double-blind, placebo controlled study. *Complementary Therapies in Medicine*, *20*(3), 124–130. https://doi.org/10.1016/j.ctim.2011.12.007

Cammisotto, P. G., Bendayan, M., Sané, A., Dominguez, M., Garofalo, C., & Levy, É (2010). Receptor-mediated transcytosis of leptin through human intestinal cells *in vitro*. *International Journal of Cell Biology*, *2010*, 13. https://doi.org/10.1155/2010/928169

Cao, Y., Miao, J., Liu, G., Luo, Z., Xia, Z., Liu, F., … Xiao, H. (2017). Bioactive peptides isolated from casein phosphopeptides enhance calcium and magnesium uptake in Caco-2 cell monolayers. *Journal of Agricultural and Food Chemistry*, *65*(11), 2307–2314. https://doi.org/10.1021/acs.jafc.6b05711

Casewell, N. R., Wagstaff, S. C., Wüster, W., Cook, D. A. N., Bolton, F. M. S., King, S. I., … Harrison, R. A. (2014). Medically important differences in snake venom composition are dictated by distinct postgenomic mechanisms. *Proceedings of the National*

Academy of Sciences, *111*(25), 9205–9210. https://doi.org/10.1073/pnas.1405484111

Chabance, B., Jolles, P., Izquierdo, C., Mazoyer, E., Francoual, C., Drouet, L., & Fiat, A. M. (1995). Characterization of an antithrombotic peptide from kappa-casein in newborn plasma after milk ingestion. *British Journal of Nutrition*, *73*(4), 583–590.

Chabance, B., Marteau, P., Rambaud, J. C., Migliore-Samour, D., Boynard, M., Perrotin, P., … Fiat, A. M. (1998). Casein peptide release and passage to the blood in humans during digestion of milk or yogurt. *Biochimie*, *80*(2), 155–165.

Chakrabarti, S., Guha, S., & Majumder, K. (2018). Food-derived bioactive peptides in human health: Challenges and opportunities. *Nutrients*, *10*(11), 1738. Retrieved from https://www.mdpi.com/2072-6643/10/11/1738

Chandrudu, S., Simerska, P., & Toth, I. (2013). Chemical methods for peptide and protein production. *Molecules (Basel, Switzerland)*, *18*(4), 4373–4388. https://doi.org/10.3390/molecules18044373

Chanphai, P., & Tajmir-Riahi, H. A. (2017). Trypsin and trypsin inhibitor bind milk beta-lactoglobulin: Protein-protein interactions and morphology. *International Journal of Biological Macromolecules*, *96*, 754–758. https://doi.org/10.1016/j.ijbiomac.2016.12.075

Chaves-López, C., Serio, A., Paparella, A., Martuscelli, M., Corsetti, A., Tofalo, R., & Suzzi, G. (2014). Impact of microbial cultures on proteolysis and release of bioactive peptides in fermented milk. *Food Microbiology*, *42*, 117–121. https://doi.org/10.1016/j.fm.2014.03.005

Chatterjee, A., Kanawjia, S. K., Khetra, Y., & Saini, P. (2015). Discordance between in silico & in vitro analyses of ACE inhibitory & antioxidative peptides from mixed milk tryptic whey protein hydrolysate. *Journal of Food Science and Technology*, *52*(9), 5621–5630. https://doi.org/10.1007/s13197-014-1669-z

Chen, J., Liu, Y., Wang, G., Sun, S., Liu, R., Hong, B., … Bai, K. (2018). Processing optimization and characterization of angiotensin-I-converting enzyme inhibitory peptides from Lizardfish (Synodus macrops) scale gelatin. *Marine Drugs*, *16*(7), 228. https://doi.org/10.3390/md16070228

Chen, L., Bao, B., Wang, N., Xie, J., & Wu, W. (2012). Oral administration of shark type II collagen suppresses complete freund's adjuvant-induced rheumatoid arthritis in rats. *Pharmaceuticals (Basel, Switzerland)*, *5*(4), 339–352. https://doi.org/10.3390/ph5040339

Cheng, S., Tu, M., Liu, H., Zhao, G., & Du, M. (2019). Food-derived antithrombotic peptides: Preparation, identification, and interactions with thrombin. *Critical Reviews in Food Science and Nutrition*, *59*(Sup1), S81–s95. https://doi.org/10.1080/10408398.2018.1524363

Choudhury, S. N., Konwar, B., Kaur, S., Doley, R., & Mondal, B. (2018). Study on snake venom protein-antibody interaction by surface plasmon resonance spectroscopy. *Photonic Sensors*, *8*(3), 193–202. https://doi.org/10.1007/s13320-018-0501-1

Cian, R. E., Martínez-Augustin, O., & Drago, S. R. (2012). Bioactive properties of peptides obtained by enzymatic hydrolysis from protein byproducts of Porphyra columbina. *Food Research International*, *49*(1), 364–372.

Conrads, T. P., Fusaro, V. A., Ross, S., Johann, D., Rajapakse, V., Hitt, B. A., … Veenstra, T. D. (2004). High-resolution serum proteomic features for ovarian cancer detection. *Endocrine-Related Cancer*, *11*(2), 163–178.

Conti, A., Fredolini, C., Tamburro, D., Magagnoli, G., Zhou, W., Liotta, L. A., … Benassi, M. S. (2016). Identification of novel candidate circulating biomarkers for malignant soft tissue sarcomas: Correlation with metastatic progression. *Proteomics*, *16*(4), 689–697. https://doi.org/10.1002/pmic.201500164

Cui, Y., Zhu, C., Ming, Z., Cao, J., Yan, Y., Zhao, P., … Chen, Q. (2017). Molecular mechanisms by which casein glycomacropeptide maintains internal homeostasis in mice with experimental ulcerative colitis. *PLoS One*, *12*(7), e0181075. https://doi.org/10.1371/journal.pone.0181075

Cushman, D. W., Cheung, H. S., Sabo, E. F., & Ondetti, M. A. (1977). Design of potent competitive inhibitors of angiotensin-converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acids. *Biochemistry*, *16*(25), 5484–5491. https://doi.org/10.1021/bi00644a014

D'Adamo, E., Giannini, C., Chiavaroli, V., Giorgis, T. d., Verrotti, A., Chiarelli, F., & Mohn, A. (2011). What is the significance of soluble and endogenous secretory receptor for advanced glycation end products in liver steatosis in obese prepubertal children? *Antioxidants & Redox Signaling*, *14*(6), 1167–1172. https://doi.org/10.1089/ars.2010.3719

Daliri, E. B.-M., Oh, D. H., & Lee, B. H. (2017). Bioactive peptides. *Foods*, *6*(5), 32. https://doi.org/10.3390/foods6050032

Damodaran, S., Wood, T. D., Nagarajan, P., & Rabin, R. A. (2007). Evaluating peptide mass fingerprinting-based protein identification. *Genomics Proteomics Bioinformatics*, *5*(3–4), 152–157. https://doi.org/10.1016/s1672-0229(08)60002-9

Dang, H. X., & Lawrence, C. B. (2014). Allerdictor: Fast allergen prediction using text classification techniques. *Bioinformatics*, *30*(8), 1120–1128. https://doi.org/10.1093/bioinformatics/btu004

Dave, L. A., Montoya, C. A., Rutherfurd, S. M., & Moughan, P. J. (2014). Gastrointestinal endogenous proteins as a source of bioactive peptides–an in silico study. *PloS one*, *9*(6), e98922–e98922. https://doi.org/10.1371/journal.pone.0098922

Deng, B., Long, H., Tang, T., Ni, X., Chen, J., Yang, G., … Yi, L. (2019). Quantitative structure-activity relationship study of antioxidant tripeptides based on model population analysis. *International Journal of Molecular Sciences*, *20*(4), 995. https://doi.org/10.3390/ijms20040995

Dia, V. P., Torres, S., De Lumen, B. O., Erdman, J. W., & Gonzalez De Mejia, E. (2009). Presence of lunasin in plasma of men after soy protein consumption. *Journal of Agricultural and Food Chemistry*, *57*(4), 1260–1266. https://doi.org/10.1021/jf803303k

Díaz-Gómez, J. L., Castorena-Torres, F., Preciado-Ortiz, R. E., & García-Lara, S. (2017). Anti-cancer activity of maize bioactive peptides. *Frontiers in Chemistry*, *5*, 44. https://doi.org/10.3389/fchem.2017.00044

Dimitrov, I., Bangov, I., Flower, D. R., & Doytchinova, I. (2014). AllerTOP v.2—A server for in silico prediction of allergens. *Journal of Molecular Modeling*, *20*(6), 2278. https://doi.org/10.1007/s00894-014-2278-5

Edwards, N. J. (2013). PepArML: A meta-search peptide identification platform for tandem mass spectra. *Current Protocols in Bioinformatics*, *44*, 13.23, 11–23. https://doi.org/10.1002/0471250953.bi1323s44

Edwards, N. J. (2017). Protein identification from tandem mass spectra by database searching. *Methods in Molecular Biology*, *1558*, 357–380. https://doi.org/10.1007/978-1-4939-6783-4_17

El-Fattah, A. M., Sakr, S. S., El-Dieb, S. M., & Elkashef, H. A. S. (2017). Bioactive peptides with ACE-I and antioxidant activity

produced from milk proteolysis. *International Journal of Food Properties*, *20*(12), 3033–3042. https://doi.org/10.1080/10942912.2016.1270963

El Hidan, M. A., Touloun, O., El Hiba, O., Laadraoui, J., Ferehan, H., & Boumezzough, A. (2016). Peripheral and central effects of intracerebroventricular microinjection of Hottentotta gentili (Pallary, 1924) (Scorpiones, Buthidae) venom. *Toxicon*, *111*, 22–30. https://doi.org/10.1016/j.toxicon.2015.12.010

Estevao-Costa, M. I., Sanz-Soler, R., Johanningmeier, B., & Eble, J. A. (2018). Snake venom components in medicine: From the symbolic rod of Asclepius to tangible medical research and application. *International Journal of Biochemistry & Cell Biology*, *104*, 94–113. https://doi.org/10.1016/j.biocel.2018.09.011

Fakhri, Y., Abtahi, M., Atamaleki, A., Raoofi, A., Atabati, H., Asadi, A., … Keramati, H. (2019). The concentration of potentially toxic elements (PTEs) in honey: A global systematic review and meta-analysis and risk assessment. *Trends in Food Science & Technology*, *98*, 498–506.

Fakhri, Y., Ghorbani, R., Taghavi, M., Keramati, H., Amanidaz, N., Moradi, B., … Khaneghah, A. M. (2019). Concentration and prevalence of aflatoxin M1 in human breast milk in Iran: Systematic review, meta-analysis, and carcinogenic risk assessment: A review. *Journal of Food Protection*, *82*(5), 785–795.

Fakhri, Y., Rahmani, J., Oliveira, C. A. F., Franco, L. T., Corassin, C. H., Saba, S., … Khaneghah, A. M. (2019). Aflatoxin M1 in human breast milk: A global systematic review, meta-analysis, and risk assessment study (Monte Carlo simulation). *Trends in Food Science & Technology*, *88*, 333–342.

Fälth, M., Sköld, K., Norrman, M., Svensson, M., Fenyö, D., & Andren, P. E. (2006). SwePep, a database designed for endogenous peptides and mass spectrometry. *Mol Cell Proteomics*, *5*(6), 998–1005. https://doi.org/10.1074/mcp.M500401-MCP200

Fan, H., Liao, W., & Wu, J. (2019). Molecular interactions, bioavailability, and cellular mechanisms of angiotensin-converting enzyme inhibitory peptides. *Journal of Food Biochemistry*, *43*(1), e12572. https://doi.org/10.1111/jfbc.12572

Fan, M., Guo, T., Li, W., Chen, J., Li, F., Wang, C., … Zhang, S. (2019). Isolation and identification of novel casein-derived bioactive peptides and potential functions in fermented casein with Lactobacillus helveticus. *Food Science and Human Wellness*, *8*(2), 156–176. https://doi.org/10.1016/j.fshw.2019.03.010

Fjell, C. D., Hancock, R. E., & Cherkasov, A. (2007). AMPer: A database and an automated discovery tool for antimicrobial peptides. *Bioinformatics*, *23*(9), 1148–1155. https://doi.org/10.1093/bioinformatics/btm068

Fu, Y., Wu, W., Zhu, M., & Xiao, Z. (2016). In silico assessment of the potential of patatin as a precursor of bioactive peptides. *Journal of Food Biochemistry*, *40*(3), 366–370. https://doi.org/10.1111/jfbc.12213

Foltz, M., van der Pijl, P. C., & Duchateau, G. S. (2010). Current *in vitro* testing of bioactive peptides is not valuable. *Journal of Nutrition*, *140*(1), 117–118. https://doi.org/10.3945/jn.109.116228

Freue, G. V. C., & Borchers, C. H. (2012). Multiple reaction monitoring (MRM). *Circulation: Cardiovascular Genetics*, *5*(3), 378–378. https://doi.org/10.1161/CIRCGENETICS.111.959528

Garcia-Vaquero, M., Mora, L., & Hayes, M. (2019). In vitro and in silico approaches to generating and identifying angiotensin-converting enzyme I inhibitory peptides from green macroalga ulva lactuca. *Mar Drugs*, *17*(4), 204. https://doi.org/10.3390/md17040204

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M., Appel, R., & Bairoch, A. (2005). John M Walker Protein identification and analysis tools on the ExPASy server. *The proteomics protocols handbook* (pp. 571–607). Totowa: Humana Press. https://archive-ouverte.unige.ch/unige:37793.

Gibbs, B. F., Zougman, A., Masse, R., & Mulligan, C. (2004). Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. *Food Research International*, *37*(2), 123–131. https://doi.org/10.1016/j.foodres.2003.09.010

Goldman, R., Ressom, H. W., Abdel-Hamid, M., Goldman, L., Wang, A., Varghese, R. S., … Moiseiwitsch, F. S. (2007). Candidate markers for the detection of hepatocellular carcinoma in low-molecular weight fraction of serum. *Carcinogenesis*, *28*(10), 2149–2153. https://doi.org/10.1093/carcin/bgm177

Griffiths, M. W., & Tellez, A. M. (2013). Lactobacillus helveticus: The proteolytic system. *Frontiers in Microbiology*, *4*, 30–30. https://doi.org/10.3389/fmicb.2013.00030

Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., Raghava, G. P. S., & Open Source Drug Discovery Consortium. (2013) In silico approach for predicting toxicity of peptides and proteins. *PLoS One*, *8*(9), e73957. https://doi.org/10.1371/journal.pone.0073957

Han, R., Maycock, J., Murray, B. S., & Boesch, C. (2019). Identification of angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory peptides derived from oilseed proteins using two integrated bioinformatic approaches. *Food Research International*, *115*, 283–291. https://doi.org/10.1016/j.foodres.2018.12.015

Hernández-Ledesma, B., Quirós, A., Amigo, L., & Recio, I. (2007). Identification of bioactive peptides after digestion of human milk and infant formula with pepsin and pancreatin. *International Dairy Journal*, *17*(1), 42–49. https://doi.org/10.1016/j.idairyj.2005.12.012

Herregods, G., Van Camp, J., Morel, N., Ghesquiere, B., Gevaert, K., Vercruysse, L., … Smagghe, G. (2011). Angiotensin I-converting enzyme inhibitory activity of gelatin hydrolysates and identification of bioactive peptides. *Journal of Agricultural and Food Chemistry*, *59*(2), 552–558. https://doi.org/10.1021/jf1037823

Hou, W., Zhang, X., & Liu, C.-F. (2017). Progress in chemical synthesis of peptides and proteins. *Transactions of Tianjin University*, *23*(5), 401–419. https://doi.org/10.1007/s12209-017-0068-8

Hou, Y., Liu, W., Cheng, Y., Zhou, J., Wu, L., & Yang, G. (2014). Production optimization and characterization of immunomodulatory peptides obtained from fermented goat placenta. *Food Science and Technology*, *34*, 723–729. Retrieved from http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20612014000400012&nrm=so

Hu, Y., & Smith, D. E. (2019). In silico prediction of the absorption and disposition of cefadroxil in humans using an intestinal permeability method scaled from humanized PepT1 mice. *Drug Metabolism & Disposition*, *47*(3), 173–183. https://doi.org/10.1124/dmd.118.084236

Ibrahim, H. R., Ahmed, A. S., & Miyata, T. (2017). Novel angiotensin-converting enzyme inhibitory peptides from caseins and whey proteins of goat milk. *Journal of Advanced Research*, *8*(1), 63–71. https://doi.org/10.1016/j.jare.2016.12.002

Ibrahim, M. A., Bester, M. J., Neitz, A. W., & Gaspar, A. R. (2018). Structural properties of bioactive peptides with α-glucosidase inhibitory activity. *Chemical Biology & Drug Design*, *91*(2), 370–379.

Ibrahim, M. A., Bester, M. J., Neitz, A. W., & Gaspar, A. R. M. (2019). Tuber storage proteins as potential precursors of bioactive peptides:

An In silico analysis. *International Journal of Peptide Research and Therapeutics*, *25*(2), 437–446. 10.1007/s10989-018-9688-7

Ichikawa, S., Morifuji, M., Ohara, H., Matsumoto, H., Takeuchi, Y., & Sato, K. (2010). Hydroxyproline-containing dipeptides and tripeptides quantified at high concentration in human blood after oral administration of gelatin hydrolysate. *International Journal of Food Sciences and Nutrition*, *61*(1), 52–60. https://doi.org/10.3109/09637480903257711

Inoue, N., Nagao, K., Sakata, K., Yamano, N., Gunawardena, P. E. R., Han, S.-Y., … Yanagita, T. (2011). Screening of soy protein-derived hypotriglyceridemic di-peptides *in vitro* and in vivo. *Lipids in Health and Disease*, *10*, 85–85. https://doi.org/10.1186/1476-511X-10-85

Iwai, K., Hasegawa, T., Taguchi, Y., Morimatsu, F., Sato, K., Nakamura, Y., … Ohtsuki, K. (2005). Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *Journal of Agricultural and Food Chemistry*, *53*(16), 6531–6536. https://doi.org/10.1021/jf050206p

Iwai, K., Zhang, Y., Kouguchi, T., Saiga-Egusa, A., Shimizu, M., Ohmori, T., … Morimatsu, F. (2009). Blood concentration of food-derived peptides following oral intake of chicken collagen hydrolysate and its angiotensin-converting enzyme inhibitory activity in healthy volunteers. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *56*(6), 326–330. https://doi.org/10.3136/nskkk.56.326

Jaradat, D. M. M. (2018). Thirteen decades of peptide synthesis: Key developments in solid phase peptide synthesis and amide bond formation utilized in peptide ligation. *Amino Acids*, *50*(1), 39–68. https://doi.org/10.1007/s00726-017-2516-0

Jauhiainen, T., Wuolle, K., Vapaatalo, H., Kerojoki, O., Nurmela, K., Lowrie, C., & Korpela, R. (2007). Oral absorption, tissue distribution and excretion of a radiolabelled analog of a milk-derived antihypertensive peptide, Ile-Pro-Pro, in rats. *International Dairy Journal*, *17*(10), 1216–1223. https://doi.org/10.1016/j.idairyj.2007.02.004

Jin, A.-H., Cristofori-Armstrong, B., Rash, L. D., Román-González, S. A., Espinosa, R. A., Lewis, R. J., … Vetter, I. (2019). Novel conorfamides from Conus austini venom modulate both nicotinic acetylcholine receptors and acid-sensing ion channels. *Biochemical Pharmacology*, *164*, 342–348. https://doi.org/10.1016/j.bcp.2019.04.025

Joel, C. H., Sutopo, C. C. Y., Prajitno, A., Su, J.-H., & Hsu, J.-L. (2018). Screening of angiotensin-I converting enzyme inhibitory peptides derived from Caulerpa lentillifera. *Molecules (Basel, Switzerland)*, *23*(11), 3005. https://doi.org/10.3390/molecules23113005

Juhász, A., Haraszi, R., & Maulis, C. (2015). ProPepper: A curated database for identification and analysis of peptide and immune-responsive epitope composition of cereal grain protein families. Database : the journal of biological databases and curation, 2015, bav100. https://doi.org/10.1093/database/bav100

Kaiser, S., Martin, M., Lunow, D., Rudolph, S., Mertten, S., Möckel, U., … Henle, T. (2016). Tryptophan-containing dipeptides are bioavailable and inhibit plasma human angiotensin-converting enzyme in vivo. *International Dairy Journal*, *52*, 107–114. https://doi.org/10.1016/j.idairyj.2015.09.004

Kalmykova, S. D., Arapidi, G. P., Urban, A. S., Osetrova, M. S., Gordeeva, V. D., Ivanov, V. T., & Govorun, V. M. (2018). In silico analysis of peptide potential biological functions. *Russian Journal of Bioorganic Chemistry*, *44*(4), 367–385. https://doi.org/10.1134/S106816201804009X

Kawaguchi, T., Nanbu, P. N., & Kurokawa, M. (2012). Distribution of prolylhydroxyproline and its metabolites after oral administration in rats. *Biological and Pharmaceutical Bulletin*, *35*(3), 422–427. https://doi.org/10.1248/bpb.35.422

Keller, J., & Layer, P. (2005). Human pancreatic exocrine response to nutrients in health and disease. *Gut*, *54*(Suppl. 6), vi1–28. https://doi.org/10.1136/gut.2005.065946

Khaneghah, A. M., Fakhri, Y., Abdi, L., Coppa, C. F. S. C., Franco, L. T., & de Oliveira, C. A. F. (2019). The concentration and prevalence of ochratoxin A in coffee and coffee-based products: A global systematic review, meta-analysis and meta-regression. *Fungal Biology*, *123*(8), 611–617.

Khaneghah, A. M., Fakhri, Y., Gahruie, H. H., Niakousari, M., & Sant'Ana, A. S. (2019). Mycotoxins in cereal-based products during 24 years (1983-2017): A global systematic review. *Trends in Food Science & Technology*, *91*, 95–105.

Khaneghah, A. M., Fakhri, Y., & Sant'Ana, A. S. (2018). Impact of unit operations during processing of cereal-based products on the levels of deoxynivalenol, total aflatoxin, ochratoxin A, and zearalenone: A systematic review and meta-analysis. *Food Chemistry*, *268*, 611–624.

Khaneghah, A. M., Kamani, M. H., Fakhri, Y., Coppa, C. F. S. C., de Oliveira, C. A. F., & Sant'Ana, A. S. (2019). Changes in masked forms of deoxynivalenol and their co-occurrence with culmorin in cereal-based products: A systematic review and meta-analysis. *Food Chemistry*, *294*, 587–596.

Khiari, Z., Ndagijimana, M., & Betti, M. (2014). Low molecular weight bioactive peptides derived from the enzymatic hydrolysis of collagen after isoelectric solubilization/precipitation process of Turkey by-products. *Poultry Science*, *93*(9), 2347–2362. https://doi.org/10.3382/ps.2014-03953

Khiari, Z., Rico, D., Martin-Diana, A. B., & Barry-Ryan, C. (2014). Structure elucidation of ACE-inhibitory and antithrombotic peptides isolated from mackerel skin gelatine hydrolysates. *Journal of the Science of Food and Agriculture*, *94*(8), 1663–1671. https://doi.org/10.1002/jsfa.6476

Kumar, R., Chaudhary, K., Sharma, M., Nagpal, G., Chauhan, J. S., Singh, S., … Raghava, G. P. (2015). AHTPDB: A comprehensive platform for analysis and presentation of antihypertensive peptides. *Nucleic Acids Res*, *43*(Database issue), D956–D962. https://doi.org/10.1093/nar/gku1141

Lafarga, T., & Hayes, M. (2017). Bioactive protein hydrolysates in the functional food ingredient industry: Overcoming current challenges. *Food Reviews International*, *33*(3), 217–246. https://doi.org/10.1080/87559129.2016.1175013

Lafarga, T., O'Connor, P., & Hayes, M. (2014). Identification of novel dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides from meat proteins using in silico analysis. *Peptides*, *59*, 53–62. https://doi.org/10.1016/j.peptides.2014.07.005

Lafarga, T., O'Connor, P., & Hayes, M. (2015). In silico methods to identify meat-derived prolyl endopeptidase inhibitors. *Food Chem*, *175*, 337–343. https://doi.org/10.1016/j.foodchem.2014.11.150

Lamberti, G., Cascone, S., Iannaccone, M., & Titomanlio, G. (2012). *in vitro* simulation of drug intestinal absorption. *International Journal of Pharmaceutics*, *439*(1), 165–168. https://doi.org/10.1016/j.ijpharm.2012.10.012

Lata, S., Mishra, N. K., & Raghava, G. P. S. (2010). AntiBP2: improved version of antibacterial peptide prediction. *BMC Bioinformatics*, *11*(1), S19. https://doi.org/10.1186/1471-2105-11-S1-S19

Lee, H. J., Jang, H. L., Ahn, D. K., Kim, H. J., Jeon, H. Y., Seo, D. B., … Kang, S. S. (2019). Orally administered collagen peptide protects against UVB-induced skin aging through the absorption of

dipeptide forms, Gly-Pro and Pro-Hyp. *Biosci Biotechnol Biochem*, *83*(6), 1146–1156. https://doi.org/10.1080/09168451.2019.1580559

Li-Chan, E. C. (2015). Bioactive peptides and protein hydrolysates: Research trends and challenges for application as nutraceuticals and functional food ingredients. *Current Opinion in Food Science*, *1*, 28–37.

Li, J., Zhang, Z., Rosenzweig, J., Wang, Y. Y., & Chan, D. W. (2002). Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. *Clinical Chemistry*, *48*(8), 1296–1304.

Liao, W., Jahandideh, F., Fan, H., Son, M., & Wu, J. (2018). Egg protein-derived bioactive peptides: Preparation, efficacy, and absorption. *Advances in Food and Nutrition Research*, *85*, 1–58. https://doi.org/10.1016/bs.afnr.2018.02.001

Lin, K., Zhang, L.-W., Han, X., Xin, L., Meng, Z.-X., Gong, P.-M., & Cheng, D.-Y. (2018). Yak milk casein as potential precursor of angiotensin I-converting enzyme inhibitory peptides based on in silico proteolysis. *Food Chemistry*, *254*, 340–347. https://doi.org/10.1016/j.foodchem.2018.02.051

Liu, Y., & Pischetsrieder, M. (2017). Identification and relative quantification of bioactive peptides sequentially released during simulated gastrointestinal digestion of commercial Kefir. *Journal of Agricultural and Food Chemistry*, *65*(9), 1865–1873. https://doi.org/10.1021/acs.jafc.6b05385

Lohner, K., & Hilpert, K. (2016). Antimicrobial peptides: Cell membrane and microbial surface interactions. *Biochimica et Biophysica Acta*, *1858*(5), 915–917.

Lomazi, R. L., Nishiduka, E. S., Silva, P. I., Jr., & Tashima, A. K. (2018). Identification of peptides in spider venom using mass spectrometry. *Methods in Molecular Biology*, *1719*, 359–367. https://doi.org/10.1007/978-1-4939-7537-2_24

Ma, S., Huang, D., Zhai, M., Yang, L., Peng, S., Chen, C., … Xu, M. (2015). Isolation of a novel bio-peptide from walnut residual protein inducing apoptosis and autophagy on cancer cells. *BMC Complementary and Alternative Medicine*, *15*, 413–413. https://doi.org/10.1186/s12906-015-0940-9

Majumder, K., & Wu, J. (2010). A new approach for identification of novel antihypertensive peptides from egg proteins by QSAR and bioinformatics. *Food Research International*, *43*(5), 1371–1378. https://doi.org/10.1016/j.foodres.2010.04.027

Manzanares, P., Gandía, M., Garrigues, S., & Marcos, J. F. (2019). Improving health-promoting effects of food-derived bioactive peptides through rational design and oral delivery strategies. *Nutrients*, *11*(10), 2545.

Marques, M. R., Cerda, A., Fontanari, G. G., Pimenta, D. C., Soares-Freitas, R. M., Hirata, M. H., … Arêas, J. A. G. (2018). Transport of cowpea bean derived peptides and their modulator effects on mRNA expression of cholesterol-related genes in Caco-2 and HepG2 cells. *Food Research International*, *107*, 165–171. https://doi.org/10.1016/j.foodres.2018.01.031

Marqus, S., Pirogova, E., & Piva, T. J. (2017). Evaluation of the use of therapeutic peptides for cancer treatment. *Journal of Biomedical Science*, *24*(1), 21.

Martorella, A., & Robbins, R. (2007). Serum peptide profiling: Identifying novel cancer biomarkers for early disease detection. *Acta BioMedica*, *78*(Suppl, 1), 123–128.

Matsui, T., Tamaya, K., Seki, E., Osajima, K., Matsumoto, K., & Kawasaki, T. (2002). Val-Tyr as a natural antihypertensive dipeptide can be absorbed into the human circulatory blood system. *Clinical and Experimental Pharmacology and Physiology*, *29*(3), 204–208. https://doi.org/10.1046/j.1440-1681.2002.03628.x

Mazorra-Manzano, M., Ramírez-Suarez, J., & Yada, R. (2018). Plant proteases for bioactive peptides release: A review. *Critical Reviews in Food Science and Nutrition*, *58*(13), 2147–2163.

Meisel, H., & Frister, H. (1989). Chemical characterization of bioactive peptides from in vivo digests of casein. *J Dairy Res*, *56*(3), 343–349.

Mennella, C., Visciano, M., Napolitano, A., Del Castillo, M. D., & Fogliano, V. (2006). Glycation of lysine-containing dipeptides. *Journal of Peptide Science*, *12*(4), 291–296. https://doi.org/10.1002/psc.722

Miao, J., Liu, G., Ke, C., Fan, W., Li, C., Chen, Y., … Xiao, H. (2016). Inhibitory effects of a novel antimicrobial peptide from kefir against Escherichia coli. *Food Control*, *65*, 63–72. https://doi.org/10.1016/j.foodcont.2016.01.023

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Dupont, D. (2014). A standardised static *in vitro* digestion method suitable for food–an international consensus. *Food & Function*, *5*(6), 1113–1124.

Minhas, G. S., & Newstead, S. (2019). Structural basis for prodrug recognition by the SLC15 family of proton-coupled peptide transporters. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(3), 804–809. https://doi.org/10.1073/pnas.1813715116

Minkiewicz, P., Dziuba, J., & Michalska, J. (2011). Bovine meat proteins as potential precursors of biologically active peptides–a computational study based on the BIOPEP database. *Food Science and Technology International*, *17*(1), 39–45. https://doi.org/10.1177/1082013210368461

Minkiewicz, P., Iwaniak, A., & Darewicz, M. (2019). BIOPEP-UWM database of bioactive peptides: Current opportunities. *International Journal of Molecular Sciences*, *20*(23), 5978. https://doi.org/10.3390/ijms20235978

Mohan, A., Udechukwu, M. C., Rajendran, S. R. C. K., & Udenigwe, C. C. (2015). Modification of peptide functionality during enzymatic hydrolysis of whey proteins. *RSC Advances*, *5*(118), 97400–97407. https://doi.org/10.1039/C5RA15140F

Mohan, N. M., Zorgani, A., Jalowicki, G., Kerr, A., Khaldi, N., & Martins, M. (2019). Unlocking NuriPep 1653 from common pea protein: A potent antimicrobial peptide to tackle a pan-drug resistant acinetobacter baumannii. *Frontiers in Microbiology*, *10*, 2086. https://doi.org/10.3389/fmicb.2019.02086

Mohayeji, M., Capriotti, A. L., Cavaliere, C., Piovesana, S., Samperi, R., Stampachiacchiere, S., … Lagana, A. (2014). Heterosis profile of sunflower leaves: A label free proteomics approach. *Journal of Proteomics*, *99*, 101–110.

Mojica, L., & de Mejía, E. G. (2016). Optimization of enzymatic production of anti-diabetic peptides from black bean (Phaseolus vulgaris L.) proteins, their characterization and biological potential. *Food & Function*, *7*(2), 713–727.

Mooney, C., Haslam, N. J., Holton, T. A., Pollastri, G., & Shields, D. C. (2013). PeptideLocator: Prediction of bioactive peptides in protein sequences. *Bioinformatics*, *29*(9), 1120–1126. https://doi.org/10.1093/bioinformatics/btt103

Mooney, C., Haslam, N. J., Pollastri, G., & Shields, D. C. (2012). Towards the improved discovery and design of functional peptides: Common features of diverse classes permit generalized prediction of bioactivity. *PLoS One*, *7*(10), e45012. https://doi.org/10.1371/journal.pone.0045012

Moronta, J., Smaldini, P. L., Fossati, C. A., Añon, M. C., & Docena, G. H. (2016). The anti-inflammatory SSEDIKE peptide from Amaranth seeds modulates IgE-mediated food allergy. *Journal of Functional Foods*, *25*, 579–587. https://doi.org/10.1016/j.jff.2016.06.031

Mourão, C. B. F., & Schwartz, E. F. (2013). Protease inhibitors from marine venomous animals and their counterparts in terrestrial venomous animals. *Marine Drugs*, *11*(6), 2069–2112. https://doi.org/10.3390/md11062069

Mulder, K., Lima, L. A., Miranda, V., Dias, S. C., & Franco, O. L. (2013). Current scenario of peptide-based drugs: The key roles of cationic antitumor and antiviral peptides. *Frontiers in Microbiology*, *4*, 321.

Mune Mune, M. A., Minka, S. R., & Henle, T. (2018). Investigation on antioxidant, angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory activity of Bambara bean protein hydrolysates. *Food Chemistry*, *250*, 162–169. https://doi.org/10.1016/j.foodchem.2018.01.001

Nagaoka, S. (2019). Structure–function properties of hypolipidemic peptides. *Journal of Food Biochemistry*, *43*(1), e12539. https://doi.org/10.1111/jfbc.12539

Najafian, L., & Babji, A. S. (2014). Production of bioactive peptides using enzymatic hydrolysis and identification antioxidative peptides from patin (Pangasius sutchi) sarcoplasmic protein hydolysate. *Journal of Functional Foods*, *9*, 280–289. https://doi.org/10.1016/j.jff.2014.05.003

Nardo, A. E., Añón, M. C., & Parisi, G. (2018). Large-scale mapping of bioactive peptides in structural and sequence space. *PLoS One*, *13*(1), e0191063–e0191063. https://doi.org/10.1371/journal.pone.0191063

Nasri, R., Amor, I. B., Bougatef, A., Nedjar-Arroume, N., Dhulster, P., Gargouri, J., … Nasri, M. (2012). Anticoagulant activities of goby muscle protein hydrolysates. *Food Chemistry*, *133*(3), 835–841.

Nii, Y., Fukuta, K., Yoshimoto, R., Sakai, K., & Ogawa, T. (2008). Determination of antihypertensive peptides from an izumi shrimp hydrolysate. *Bioscience, Biotechnology, and Biochemistry*, *72*(3), 861–864. https://doi.org/10.1271/bbb.70565

Nishikimi, A., Koyama, Y. I., Ishihara, S., Kobayashi, S., Tometsuka, C., Kusubata, M., … Katagiri, K. (2018). Collagen-derived peptides modulate CD4(+) T-cell differentiation and suppress allergic responses in mice. *Immunity, Inflammation and Disease*, *6*(2), 245–255. https://doi.org/10.1002/iid3.213

Nongonierma, A. B., & FitzGerald, R. J. (2014). An in silico model to predict the potential of dietary proteins as sources of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides. *Food Chemistry*, *165*, 489–498.

Nongonierma, A. B., & FitzGerald, R. J. (2017). Strategies for the discovery and identification of food protein-derived biologically active peptides. *Trends in Food Science & Technology*, *69*, 289–305.

Nongonierma, A. B., Paolella, S., Mudgil, P., Maqsood, S., & FitzGerald, R. J. (2017). Dipeptidyl peptidase IV (DPP-IV) inhibitory properties of camel milk protein hydrolysates generated with trypsin. *Journal of Functional Foods*, *34*, 49–58. https://doi.org/10.1016/j.jff.2017.04.016

Offret, C., Fliss, I., Bazinet, L., Marette, A., & Beaulieu, L. (2019). Identification of a novel antibacterial peptide from Atlantic Mackerel belonging to the GAPDH-related antimicrobial family and its *in vitro* digestibility. *Marine Drugs*, *17*(7), 413. https://doi.org/10.3390/md17070413

Ohara, H., Matsumoto, H., Ito, K., Iwai, K., & Sato, K. (2007). Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *Journal of Agricultural and Food Chemistry*, *55*(4), 1532–1535. https://doi.org/10.1021/jf062834s

Orona-Tamayo, D., Valverde, M. E., & Paredes-Lopez, O. (2019). Bioactive peptides from selected Latin American food crops - A nutraceutical and molecular approach. *Critical Reviews in Food Science and Nutrition*, *59*, 1949–1975. https://doi.org/10.1080/10408398.2018.1434480

Pabst, O., & Mowat, A. M. (2012). Oral tolerance to food protein. *Mucosal Immunology*, *5*, 232. https://doi.org/10.1038/mi.2012.4

Pangestuti, R., & Kim, S.-K. (2017). Bioactive peptide of marine origin for the prevention and treatment of non-communicable diseases. *Marine Drugs*, *15*(3), 67.

Park, Y. W., & Nam, M. S. (2015). Bioactive peptides in milk and dairy products: A review. *Korean Journal for Food Science of Animal Resources*, *35*(6), 831.

Pennington, M. W., Czerwinski, A., & Norton, R. S. (2018). Peptide therapeutics from venom: Current status and potential. *Bioorganic & Medicinal Chemistry*, *26*(10), 2738–2758. https://doi.org/10.1016/j.bmc.2017.09.029

Petras, D., Hempel, B. F., Gocmen, B., Karis, M., Whiteley, G., Wagstaff, S. C., … Sussmuth, R. D. (2019). Intact protein mass spectrometry reveals intraspecies variations in venom composition of a local population of Vipera kaznakovi in Northeastern Turkey. *Journal of Proteomics*, *199*, 31–50. https://doi.org/10.1016/j.jprot.2019.02.004

Piovesana, S., Capriotti, A. L., Cavaliere, C., La Barbera, G., Montone, C. M., Chiozzi, R. Z., & Laganà, A. (2018). Recent trends and analytical challenges in plant bioactive peptide separation, identification and validation. *Analytical and Bioanalytical Chemistry*, *410*(15), 3425–3444.

Popay, J., Roberts, H., Sowden, A., Petticrew, M., Arai, L., Rodgers, M., … Duffy, S. (2006). Guidance on the conduct of narrative synthesis in systematic reviews. *A Product from the ESRC Methods Programme Version*, *1*, b92.

Power, O., Jakeman, P., & FitzGerald, R. (2013). Antioxidative peptides: Enzymatic production, *in vitro* and *in vivo* antioxidant activity and potential applications of milk-derived antioxidative peptides. *Amino acids*, *44*(3), 797–820.

Qin, Y., Qin, Z. D., Chen, J., Cai, C. G., Li, L., Feng, L. Y., … Luo, X. F. (2019). From antimicrobial to anticancer peptides: The transformation of peptides. *Recent Patients on Anticancer Drug Discovery*, *14*(1), 70–84. https://doi.org/10.2174/1574892814666190119165157

Räder, A. F. B., Reichart, F., Weinmüller, M., & Kessler, H. (2018). Improving oral bioavailability of cyclic peptides by N-methylation. *Bioorganic & Medicinal Chemistry*, *26*(10), 2766–2773. https://doi.org/10.1016/j.bmc.2017.08.031

Rafiq, S., Huma, N., Pasha, I., Shahid, M., & Xiao, H. (2017). Angiotensin-converting enzyme-inhibitory and antithrombotic activities of soluble peptide extracts from buffalo and cow milk Cheddar cheeses. *International Journal of Dairy Technology*, *70*(3), 380–388. https://doi.org/10.1111/1471-0307.12373

Ramirez, L., Shekhtman, A., & Pande, J. (2018). Nuclear magnetic resonance-based structural characterization and backbone dynamics of recombinant bee venom melittin. *Biochemistry*, *57*(19), 2775–2785. https://doi.org/10.1021/acs.biochem.8b00156

Rani, S., Pooja, K., & Pal, G. K. (2017). Exploration of potential angiotensin converting enzyme inhibitory peptides generated from enzymatic hydrolysis of goat milk proteins. *Biocatalysis and*

Agricultural Biotechnology, *11*, 83–88. https://doi.org/10.1016/j.bcab.2017.06.008

Rani, S., Pooja, K., & Pal, G. K. (2018). Exploration of rice protein hydrolysates and peptides with special reference to antioxidant potential: Computational derived approaches for bio-activity determination. *Trends in Food Science & Technology*, *80*, 61–70. https://doi.org/10.1016/j.tifs.2018.07.013

Rashid, R., Veleba, M., & Kline, K. A. (2016). Focal targeting of the bacterial envelope by antimicrobial peptides. *Frontiers in Cell and Developmental Biology*, *4*, 55.

Raveschot, C., Cudennec, B., Coutte, F., Flahaut, C., Fremont, M., Drider, D., & Dhulster, P. (2018). Production of bioactive peptides by lactobacillus species: From gene to application. *Frontiers in Microbiology*, *9*, 2354–2354. https://doi.org/10.3389/fmicb.2018.02354

Raz, R., Burlina, F., Ismail, M., Downward, J., Li, J., Smerdon, S. J., … Offer, J. (2016). HF-free Boc synthesis of peptide thioesters for ligation and cyclization. *Angewandte Chemie*, *55*(42), 13174–13179. https://doi.org/10.1002/anie.201607657

Reche, P. A., Zhang, H., Glutting, J. P., & Reinherz, E. L. (2005). EPIMHC: A curated database of MHC-binding peptides for customized computational vaccinology. *Bioinformatics*, *21*(9), 2140-2141. https://doi.org/10.1093/bioinformatics/bti269

Ressom, H. W., Varghese, R. S., Goldman, L., An, Y., Loffredo, C. A., Abdel-Hamid, M., … Goldman, R. (2008). Analysis of MALDI-TOF mass spectrometry data for discovery of peptide and glycan biomarkers of hepatocellular carcinoma. *Journal of Proteome Research*, *7*(2), 603–610. https://doi.org/10.1021/pr0705237

Riedl, S., Leber, R., Rinner, B., Schaider, H., Lohner, K., & Zweytick, D. (2015). Human lactoferricin derived di-peptides deploying loop structures induce apoptosis specifically in cancer cells through targeting membranous phosphatidylserine. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1848*(11), 2918–2931.

Riedl, S., Rinner, B., Asslaber, M., Schaider, H., Walzer, S., Novak, A., … Zweytick, D. (2011). In search of a novel target—Phosphatidylserine exposed by non-apoptotic tumor cells and metastases of malignancies with poor treatment efficacy. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1808*(11), 2638–2645.

Rizzello, C. G., Lorusso, A., Russo, V., Pinto, D., Marzani, B., & Gobbetti, M. (2017). Improving the antioxidant properties of quinoa flour through fermentation with selected autochthonous lactic acid bacteria. *International Journal of Food Microbiology*, *241*, 252–261. https://doi.org/10.1016/j.ijfoodmicro.2016.10.035

Rizzello, C. G., Tagliazucchi, D., Babini, E., Rutella, G. S., Saa, D. L. T., & Gianotti, A. (2016). Bioactive peptides from vegetable food matrices: Research trends and novel biotechnologies for synthesis and recovery. *Journal of Functional Foods*, *27*, 549–569.

Roldan, N. R., Jimenez, M., Cervantes-Garcia, D., Marin, E., & Salinas, E. (2016). Glycomacropeptide administration attenuates airway inflammation and remodeling associated to allergic asthma in rat. *Inflammation Research*, *65*(4), 273–283. https://doi.org/10.1007/s00011-015-0913-y

Rutherfurd-Markwick, K. J. (2012). Food proteins as a source of bioactive peptides with diverse functions. *British Journal of Nutrition*, *108*(Suppl, 2), S149–157. https://doi.org/10.1017/s000711451200253x

Saha, S., & Raghava, G. P. (2006). AlgPred: Prediction of allergenic proteins and mapping of IgE epitopes. *Nucleic Acids Research*, *34*(Web Server issue), W202–209. https://doi.org/10.1093/nar/gkl343

Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. *Food Quality and Safety*, *1*(1), 29–46. https://doi.org/10.1093/fqsafe/fyx006

Saraswathy, N., & Ramalingam, P. (2011). 13 - Protein identification by peptide mass fingerprinting (PMF). In N. Saraswathy & P. Ramalingam (Eds.), *Concepts and techniques in genomics and proteomics* (pp. 185–192). Oxford: Woodhead Publishing.

Sato, K. (2017). The presence of food-derived collagen peptides in human body-structure and biological activity. *Food & Function*, *8*(12), 4325–4330. https://doi.org/10.1039/c7fo01275f

Sato, M., Hosokawa, T., Yamaguchi, T., Nakano, T., Muramoto, K., Kahara, T., … Nakano, T. (2002). Angiotensin I-converting enzyme inhibitory peptides derived from wakame (Undaria pinnatifida) and their antihypertensive effect in spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry*, *50*(21), 6245–6252. https://doi.org/10.1021/jf020482t

Sayd, T., Dufour, C., Chambon, C., Buffière, C., Remond, D., & Santé-Lhoutellier, V. (2018). Combined in vivo and in silico approaches for predicting the release of bioactive peptides from meat digestion. *Food Chemistry*, *249*, 111–118. https://doi.org/10.1016/j.foodchem.2018.01.013

Sayers, E. W., Beck, J., Brister, J. R., Bolton, E. E., Canese, K., Comeau, D. C., … Ostell, J. (2019). Database resources of the national center for biotechnology information. *Nucleic Acids Research*, *48*(D1), D9–D16. https://doi.org/10.1093/nar/gkz899

Sentandreu, M. A., & Toldra, F. (2006). A fluorescence-based protocol for quantifying angiotensin-converting enzyme activity. *Nature Protocols*, *1*(5), 2423–2427. https://doi.org/10.1038/nprot.2006.349

Shen, W., & Matsui, T. (2017). Current knowledge of intestinal absorption of bioactive peptides. *Food & Function*, *8*(12), 4306–4314. https://doi.org/10.1039/c7fo01185g

Shen, W., & Matsui, T. (2019). Intestinal absorption of small peptides: A review. *International Journal of Food Science & Technology*, *54*(6), 1942–1948. https://doi.org/10.1111/ijfs.14048

Shi, L., Zhang, Q., Rui, W., Lu, M., Jing, X., Shang, T., & Tang, J. (2004). BioPD: a web-based information center for bioactive peptides. *Regul Pept*, *120*(1–3), 1–3. https://doi.org/10.1016/j.regpep.2004.03.002

Shigemura, Y., Akaba, S., Kawashima, E., Park, E. Y., Nakamura, Y., & Sato, K. (2011). Identification of a novel food-derived collagen peptide, hydroxyprolyl-glycine, in human peripheral blood by pre-column derivatisation with phenyl isothiocyanate. *Food Chemistry*, *129*(3), 1019–1024. https://doi.org/10.1016/j.foodchem.2011.05.066

Shigemura, Y., Iwai, K., Morimatsu, F., Iwamoto, T., Mori, T., Oda, C., … Sato, K. (2009). Effect of Prolyl-hydroxyproline (Pro-Hyp), a food-derived collagen peptide in human blood, on growth of fibroblasts from mouse skin. *Journal of Agricultural and Food Chemistry*, *57*(2), 444–449. https://doi.org/10.1021/jf802785h

Shigemura, Y., Kubomura, D., Sato, Y., & Sato, K. (2014). Dose-dependent changes in the levels of free and peptide forms of hydroxyproline in human plasma after collagen hydrolysate ingestion. *Food Chemistry*, *159*, 328–332. https://doi.org/10.1016/j.foodchem.2014.02.091

Shigemura, Y., Nakaba, M., Shiratsuchi, E., Suyama, M., Yamada, M., Kiyono, T., … Sato, K. (2012). Identification of food-derived elastin peptide, prolyl-glycine (Pro-Gly), in human blood after ingestion of elastin hydrolysate. *Journal of Agricultural and Food Chemistry*, *60*(20), 5128–5133. https://doi.org/10.1021/jf300497p

Shigemura, Y., Suzuki, A., Kurokawa, M., Sato, Y., & Sato, K. (2018). Changes in composition and content of food-derived peptide in human blood after daily ingestion of collagen hydrolysate for 4 weeks. *Journal of the Science of Food and Agriculture*, *98*(5), 1944–1950. https://doi.org/10.1002/jsfa.8677

Shtatland, T., Guettler, D., Kossodo, M., Pivovarov, M., & Weissleder, R. (2007). PepBank - a database of peptides based on sequence text mining and public peptide data sources. *BMC Bioinformatics*, *8*(1), 280. https://doi.org/10.1186/1471-2105-8-280

Singhal, N., Kumar, M., Kanaujia, P. K., & Virdi, J. S. (2015). MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, *6*, 791. https://doi.org/10.3389/fmicb.2015.00791

Sontakke, S. B., Jung, J.-H., Piao, Z., & Chung, H. J. (2016). Orally available collagen tripeptide: Enzymatic stability, intestinal permeability, and absorption of Gly-Pro-Hyp and Pro-Hyp. *Journal of Agricultural and Food Chemistry*, *64*(38), 7127–7133. https://doi.org/10.1021/acs.jafc.6b02955

Srinivas, S., & Prakash, V. (2010). Bioactive peptides from bovine milk α-casein: Isolation, characterization and multifunctional properties. *International Journal of Peptide Research and Therapeutics*, *16*(1), 7–15.

Su, L.-Y., Shi, Y.-X., Yan, M.-R., Xi, Y., & Su, X.-L. (2015). Anticancer bioactive peptides suppress human colorectal tumor cell growth and induce apoptosis via modulating the PARP-p53-Mcl-1 signaling pathway. *Acta Pharmacologica Sinica*, *36*(12), 1514–1519. https://doi.org/10.1038/aps.2015.80

Sugihara, F., Inoue, N., Kuwamori, M., & Taniguchi, M. (2012). Quantification of hydroxyprolylglycine (Hyp-Gly) in human blood after ingestion of collagen hydrolysate. *Journal of Bioscience and Bioengineering*, *113*(2), 202–203. https://doi.org/10.1016/j.jbiosc.2011.09.024

Sultan, S., Huma, N., Butt, M. S., Aleem, M., & Abbas, M. (2018). Therapeutic potential of dairy bioactive peptides: A contemporary perspective. *Critical Reviews in Food Science and Nutrition*, *58*(1), 105–115.

Svedberg, J., de Haas, J., Leimenstoll, G., Paul, F., & Teschemacher, H. (1985). Demonstration of beta-casomorphin immunoreactive materials in *in vitro* digests of bovine milk and in small intestine contents after bovine milk ingestion in adult humans. *Peptides*, *6*(5), 825–830.

Taga, Y., Kusubata, M., Ogawa-Goto, K., & Hattori, S. (2014). Highly accurate quantification of hydroxyproline-containing peptides in blood using a protease digest of stable isotope-labeled collagen. *Journal of Agricultural and Food Chemistry*, *62*(50), 12096–12102. https://doi.org/10.1021/jf5039597

Taga, Y., Kusubata, M., Ogawa-Goto, K., & Hattori, S. (2016). Efficient absorption of X-Hydroxyproline (Hyp)-Gly after oral administration of a novel gelatin hydrolysate prepared using ginger protease. *Journal of Agricultural and Food Chemistry*, *64*(14), 2962–2970. https://doi.org/10.1021/acs.jafc.6b00609

Taga, Y., Kusubata, M., Ogawa-Goto, K., & Hattori, S. (2017). Identification of collagen-derived hydroxyproline (Hyp)-containing cyclic dipeptides with high oral bioavailability: Efficient formation of Cyclo(X-Hyp) from X-Hyp-Gly-type tripeptides by heating. *Journal of Agricultural and Food Chemistry*, *65*(43), 9514–9521. https://doi.org/10.1021/acs.jafc.7b03714

Taga, Y., Iwasaki, Y., Shigemura, Y., & Mizuno, K. (2019). Improved in vivo tracking of orally administered collagen hydrolysate using stable isotope labeling and LC–MS techniques. *Journal of Agricultural and Food Chemistry*, *67*(16), 4671–4678. https://doi.org/10.1021/acs.jafc.9b00571

Théolier, J., Fliss, I., Jean, J., & Hammami, R. (2014). MilkAMP: A comprehensive database of antimicrobial peptides of dairy origin. *Dairy Science & Technology*, *94*(2), 181–193. https://doi.org/10.1007/s13594-013-0153-2

Ten Have, G. A. M., van der Pijl, P. C., Kies, A. K., & Deutz, N. E. P. (2015). Enhanced lacto-tri-peptide bio-availability by co-ingestion of macronutrients. *PloS One*, *10*(6), e0130638. https://doi.org/10.1371/journal.pone.0130638

Tiefenbrunn, T. K., & Dawson, P. E. (2010). Chemoselective ligation techniques: Modern applications of time-honored chemistry. *Biopolymers*, *94*(1), 95–106. https://doi.org/10.1002/bip.21337

Tirumalai, R. S., Chan, K. C., Prieto, D. A., Issaq, H. J., Conrads, T. P., & Veenstra, T. D. (2003). Characterization of the low molecular weight human serum proteome. *Molecular & Cellular Proteomics*, *2*(10), 1096–1103. https://doi.org/10.1074/mcp.M300031-MCP200

Toll, H., Oberacher, H., Swart, R., & Huber, C. G. (2005). Separation, detection, and identification of peptides by ion-pair reversed-phase high-performance liquid chromatography-electrospray ionization mass spectrometry at high and low pH. *Journal of Chromatography A*, *1079*(1–2), 274–286.

Trc, T., & Bohmova, J. (2011). Efficacy and tolerance of enzymatic hydrolysed collagen (EHC) vs. glucosamine sulphate (GS) in the treatment of knee osteoarthritis (KOA). *International Orthopaedics*, *35*(3), 341–348. https://doi.org/10.1007/s00264-010-1010-z

Tu, M., Cheng, S., Lu, W., & Du, M. (2018). Advancement and prospects of bioinformatics analysis for studying bioactive peptides from food-derived protein: Sequence, structure, and functions. *TrAC Trends in Analytical Chemistry*, *105*, 7–17. https://doi.org/10.1016/j.trac.2018.04.005

Tu, M., Feng, L., Wang, Z., Qiao, M., Shahidi, F., Lu, W., & Du, M. (2017). Sequence analysis and molecular docking of antithrombotic peptides from casein hydrolysate by trypsin digestion. *Journal of Functional Foods*, *32*, 313–323. https://doi.org/10.1016/j.jff.2017.03.015

Tu, M., Liu, H., Cheng, S., Mao, F., Chen, H., Fan, F., … Du, M. (2019). Identification and characterization of a novel casein anticoagulant peptide derived from in vivo digestion. *Food & Function*, *10*(5), 2552–2559. https://doi.org/10.1039/C8FO02546K

Tu, M., Liu, H., Zhang, R., Chen, H., Mao, F., Cheng, S., … Du, M. (2018). Analysis and evaluation of the inhibitory mechanism of a novel angiotensin-I-converting enzyme inhibitory peptide derived from casein hydrolysate. *Journal of Agricultural and Food Chemistry*, *66*(16), 4139–4144. https://doi.org/10.1021/acs.jafc.8b00732

Tu, M., Wang, C., Chen, C., Zhang, R., Liu, H., Lu, W., … Du, M. (2018). Identification of a novel ACE-inhibitory peptide from casein and evaluation of the inhibitory mechanisms. *Food Chemistry*, *256*, 98–104. https://doi.org/10.1016/j.foodchem.2018.02.107

Tulipano, G., Faggi, L., Nardone, A., Cocchi, D., & Caroli, A. M. (2015). Characterisation of the potential of β-lactoglobulin and α-lactalbumin as sources of bioactive peptides affecting incretin function: In silico and in vitro comparative studies. *International Dairy Journal*, *48*, 66–72. https://doi.org/10.1016/j.idairyj.2015.01.008

Udenigwe, C. C. (2016). Towards rice bran protein utilization: In silico insight on the role of oryzacystatins in biologically-active peptide production. *Food Chemistry*, *191*, 135–138. https://doi.org/10.1016/j.foodchem.2015.01.043

Udenigwe, C. C., & Aluko, R. E. (2012). Food protein-derived bioactive peptides: Production, processing, and potential health benefits. *Journal of Food Science*, *77*(1), R11–24. https://doi.org/10.1111/j.1750-3841.2011.02455.x

Udenigwe, C. C., & Fogliano, V. (2017). Food matrix interaction and bioavailability of bioactive peptides: Two faces of the same coin? *Journal of Functional Foods*, *35*, 9–12. https://doi.org/10.1016/j.jff.2017.05.029

Udenigwe, C. C., Gong, M., & Wu, S. (2013). In silico analysis of the large and small subunits of cereal RuBisCO as precursors of cryptic bioactive peptides. *Process Biochemistry*, *48*(11), 1794–1799. https://doi.org/10.1016/j.procbio.2013.08.013

van der Wel, T., Janssen, F. J., Baggelaar, M. P., Deng, H., den Dulk, H., Overkleeft, H. S., & van der Stelt, M. (2015). A natural substrate-based fluorescence assay for inhibitor screening on diacylglycerol lipase α. *Journal of Lipid Research*, *56*(4), 927–935. https://doi.org/10.1194/jlr.D056390

Van Lancker, F., Adams, A., & De Kimpe, N. (2011). Chemical modifications of peptides and their impact on food properties. *Chemical Reviews*, *111*(12), 7876–7903. https://doi.org/10.1021/cr200032j

Vanier, G. S. (2013). Microwave-assisted solid-phase peptide synthesis based on the Fmoc protecting group strategy (CEM). *Methods in Molecular Biology*, *1047*, 235–249. https://doi.org/10.1007/978-1-62703-544-6_17

Vijayakumar, V., Guerrero, A. N., Davey, N., Lebrilla, C. B., Shields, D. C., & Khaldi, N. (2012). EnzymePredictor: A tool for predicting and visualizing enzymatic cleavages of digested proteins. *Journal of Proteome Research*, *11*(12), 6056–6065. https://doi.org/10.1021/pr300721f

Villanueva, J., Philip, J., Entenberg, D., Chaparro, C. A., Tanwar, M. K., Holland, E. C., & Tempst, P. (2004). Serum peptide profiling by magnetic particle-assisted, automated sample processing and MALDI-TOF mass spectrometry. *Analytical Chemistry*, *76*(6), 1560–1570. https://doi.org/10.1021/ac0352171

Villaseñor, R., Lampe, J., Schwaninger, M., & Collin, L. (2019). Intracellular transport and regulation of transcytosis across the blood–brain barrier. *Cellular and Molecular Life Sciences*, *76*(6), 1081–1092. https://doi.org/10.1007/s00018-018-2982-x

Vorherr, T., Lewis, I., Berghausen, J., Desrayaud, S., & Schaefer, M. (2018). Modulation of oral bioavailability and metabolism for closely related cyclic hexapeptides. *International Journal of Peptide Research and Therapeutics*, *24*(1), 35–48. https://doi.org/10.1007/s10989-017-9590-8

Wada, Y., Phinney, B. S., Weber, D., & Lönnerdal, B. (2017). In vivo digestomics of milk proteins in human milk and infant formula using a suckling rat pup model. *Peptides*, *88*, 18–31. https://doi.org/10.1016/j.peptides.2016.11.012

Waghu, F. H., Barai, R. S., Gurung, P., & Idicula-Thomas, S. (2016). CAMPR3: A database on sequences, structures and signatures of antimicrobial peptides. *Nucleic Acids Research*, *44*(D1), D1094–1097. https://doi.org/10.1093/nar/gkv1051

Mohtar Wan, W., Hamid, A. A., Abd-Aziz, S., Muhamad, S. K. S., & Saari, N. (2014). Preparation of bioactive peptides with high angiotensin converting enzyme inhibitory activity from winged bean [Psophocarpus tetragonolobus (L.) DC.] seed. *Journal of Food Science and Technology*, *51*(12), 3658–3668. https://doi.org/10.1007/s13197-012-0919-1

Wang, G., Li, X., & Wang, Z. (2016). APD3: The antimicrobial peptide database as a tool for research and education. *Nucleic Acids Research*, *44*(D1), D1087–1093. https://doi.org/10.1093/nar/gkv1278

Wang, J.-H., Liu, Y.-L., Ning, J.-H., Yu, J., Li, X.-H., & Wang, F.-X. (2013). Is the structural diversity of tripeptides sufficient for developing functional food additives with satisfactory multiple bioactivities? *Journal of Molecular Structure*, *1040*, 164–170.

Wang, L., Dong, C., Li, X., Han, W., & Su, X. (2017). Anticancer potential of bioactive peptides from animal sources (Review). *Oncology Reports*, *38*(2), 637–651. https://doi.org/10.3892/or.2017.5778

Wang, X., Yu, H., Xing, R., & Li, P. (2017). Characterization, preparation, and purification of marine bioactive peptides. *BioMed Research International*, *2017*, 16. https://doi.org/10.1155/2017/9746720

Wang, Y., Wang, M., Yin, S., Jang, R., Wang, J., Xue, Z., & Xu, T. (2015). NeuroPep: A comprehensive resource of neuropeptides. *Database: The Journal of Biological Databases and Curation*, *2015*, bav038–bav038. https://doi.org/10.1093/database/bav038

Watanabe-Kamiyama, M., Shimizu, M., Kamiyama, S., Taguchi, Y., Sone, H., Morimatsu, F., … Komai, M. (2010). Absorption and effectiveness of orally administered low molecular weight collagen hydrolysate in rats. *Journal of Agricultural and Food Chemistry*, *58*(2), 835–841. https://doi.org/10.1021/jf9031487

Wattanasiritham, L., Theerakulkait, C., Wickramasekara, S., Maier, C. S., & Stevens, J. F. (2016). Isolation and identification of antioxidant peptides from enzymatically hydrolyzed rice bran protein. *Food Chemistry*, *192*, 156–162. https://doi.org/10.1016/j.foodchem.2015.06.057

Weimann, C., Meisel, H., & Erhardt, G. (2009). Short communication: Bovine κ-casein variants result in different angiotensin I converting enzyme (ACE) inhibitory peptides. *Journal of Dairy Science*, *92*(5), 1885–1888. https://doi.org/10.3168/jds.2008-1671

Wen, M., & Jin, Y. (2017). A comparative analysis of human plasma and serum proteins by combining native PAGE, whole-gel slicing and quantitative LC-MS/MS: Utilizing native MS-electropherograms in proteomic analysis for discovering structure and interaction-correlated differences. *Electrophoresis*, *38*(24), 3111–3123. https://doi.org/10.1002/elps.201700261

Whelan, R. S., Konstantinidis, K., Wei, A.-C., Chen, Y., Reyna, D. E., Jha, S., … Thompson, C. B. (2012). Bax regulates primary necrosis through mitochondrial dynamics. *Proceedings of the National Academy of Sciences*, *109*(17), 6566–6571.

Wiener, A., Shudler, M., Levit, A., & Niv, M. Y. (2012). BitterDB: A database of bitter compounds. *Nucleic Acids Research*, *40*, D413–D419. https://doi.org/10.1093/nar/gkr755

Wigren, M., Bjorkbacka, H., Andersson, L., Ljungcrantz, I., Fredrikson, G. N., Persson, M., … Nilsson, J. (2012). Low levels of circulating CD4+FoxP3+ T cells are associated with an increased risk for development of myocardial infarction but not for stroke. *Arterioscler, Thrombosis, and Vascular Biology*, *32*(8), 2000–2004. https://doi.org/10.1161/atvbaha.112.251579

Wu, J., Aluko, R. E., & Nakai, S. (2006). Structural requirements of angiotensin I-converting enzyme inhibitory peptides: Quantitative structure−activity relationship study of di-and tripeptides. *Journal of Agricultural and Food Chemistry*, *54*(3), 732–738.

Xi, C., Tan, L., Sun, Y., Liang, F., Liu, N., Xue, H., … Xi, Y. (2009). A novel recombinant peptide containing only two T-cell tolerance epitopes of chicken type II collagen that suppresses collagen-induced

arthritis. *Molecular Immunology*, *46*(4), 729–737. https://doi.org/10.1016/j.molimm.2008.10.016

Xie, C.-L., Kim, J.-S., Ha, J.-M., Choung, S.-Y., & Choi, Y.-J. (2014). Angiotensin I-converting enzyme inhibitor derived from cross-linked oyster protein. *BioMed Research International*, *2014*, 379234–379234. https://doi.org/10.1155/2014/379234

Xu, Q., Hong, H., Wu, J., & Yan, X. (2019). Bioavailability of bioactive peptides derived from food proteins across the intestinal epithelial membrane: A review. *Trends in Food Science & Technology*, *86*, 399–411. https://doi.org/10.1016/j.tifs.2019.02.050

Yamamoto, S., Deguchi, K., Onuma, M., Numata, N., & Sakai, Y. (2016). Absorption and urinary excretion of peptides after collagen tripeptide ingestion in humans. *Biological and Pharmaceutical Bulletin*, *39*(3), 428–434. https://doi.org/10.1248/bpb.b15-00624

Yang, X., Chen, K., Liu, H., Zhang, Y., & Luo, Y. (2019). Purification and identification of peptides with high angiotensin-I converting enzyme (ACE) inhibitory activity from honeybee pupae (Apis mellifera) hydrolysates with in silico gastrointestinal digestion. *European Food Research and Technology*, *245*(3), 535–544. https://doi.org/10.1007/s00217-018-03223-7

Yang, X., Sheng, J., Chen, J., Peng, T., Yin, X., Liu, W., … Yang, X. (2019). The effect of Longan Arillus extract on enhancing oral absorption of bioactive peptides derived from defatted walnut meal hydrolysates. *Journal of Functional Foods*, *57*, 309–316. https://doi.org/10.1016/j.jff.2019.04.018

Yasufuku, K., Heidler, K. M., O'Donnell, P. W., Smith, G. N., Jr., Cummings, O. W., Foresman, B. H., … Wilkes, D. S. (2001). Oral tolerance induction by type V collagen downregulates lung allograft rejection. *American Journal of Respiratory Cell and Molecular Biology*, *25*(1), 26–34. https://doi.org/10.1165/ajrcmb.25.1.4431

Yazaki, M., Ito, Y., Yamada, M., Goulas, S., Teramoto, S., Nakaya, M.-a., … Yamaguchi, K. (2017). Oral ingestion of collagen hydrolysate leads to the transportation of highly concentrated Gly-Pro-Hyp and its hydrolyzed form of Pro-Hyp into the bloodstream and skin. *Journal of Agricultural and Food Chemistry*, *65*(11), 2315–2322. https://doi.org/10.1021/acs.jafc.6b05679

Ye, N., Hu, P., Xu, S., Chen, M., Wang, S., Hong, J., … Cai, T. (2018). Preparation and characterization of antioxidant peptides from carrot seed protein. *Journal of Food Quality*, *2018*, 1–9.

Yin, L., Huai, Y., Zhao, C., Ding, H., Jiang, T., & Shi, Z. (2018). Early second-trimester peptidomic identification of serum peptides for potential prediction of gestational diabetes mellitus. *Cellular Physiology and Biochemistry*, *51*(3), 1264–1275. https://doi.org/10.1159/000495538

Yu, G., Wang, F., Zhang, B., & Fan, J. (2016). *in vitro* inhibition of platelet aggregation by peptides derived from oat (Avena sativa L.), highland barley (Hordeum vulgare Linn. var. nudum Hook. f.), and buckwheat (Fagopyrum esculentum Moench) proteins. *Food Chemistry*, *194*, 577–586. https://doi.org/10.1016/j.foodchem.2015.08.058

Yvon, M., & Pelissier, J. P. (1987). Characterization and kinetics of evacuation of peptides resulting from casein hydrolysis in the stomach of the calf. *Journal of Agricultural and Food Chemistry*, *35*(1), 148–156.

Zamyatnin, A. A., Borchikov, A. S., Vladimirov, M. G., & Voronina, O. L. (2006). The EROP-Moscow oligopeptide database. *Nucleic Acids Res*, *34*(Database issue), D261–266. https://doi.org/10.1093/nar/gkj008

Zhang, L., Huang, Y., Zou, Z., He, Y., Chen, X., & Tao, A. (2012). SORTALLER: predicting allergens using substantially optimized algorithm on allergen family featured peptides. *Bioinformatics*, *28*(16), 2178–2179. https://doi.org/10.1093/bioinformatics/bts326

Zhang, Y., Shen, Y., Zhang, H., Wang, L., Zhang, H., Qian, H., & Qi, X. (2018). Isolation, purification and identification of two antioxidant peptides from water hyacinth leaf protein hydrolysates (WHLPH). *European Food Research and Technology*, *244*(1), 83–96.

Zhou, J., Yang, X., Zhang, W., Wang, J., Wei, C., Gu, F., … Qin, Y. (2017). Construction of an Anticancer Fusion Peptide (ACFP) derived from milk proteins and an assay of anti-ovarian cancer cells *in vitro*. *Anti-Cancer Agents in Medicinal Chemistry*, *17*(4), 635–643. https://doi.org/10.2174/1871520616666160627091131

Zhu, B., He, H., & Hou, T. (2019). A comprehensive review of corn protein-derived bioactive peptides: Production, characterization, bioactivities, and transport pathways. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 329–345. https://doi.org/10.1111/1541-4337.12411

Zou, T.-B., He, T.-P., Li, H.-B., Tang, H.-W., & Xia, E.-Q. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules (Basel, Switzerland)*, *21*(1), 72.

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